JOURNAL OF DAIRY SCIENCE

OFFICIAL PUBLICATION OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION SINCE 1917

E. O. HERREID, Editor-in-Chief, University of Illinois
Editorial Address: Station A, Box 250, Champaign, Illinois
Phones: 356-7073 and 333-4440 Area Code 217

EDITORIAL BOARD

U. S. Ashworth
Pullman, Washington

M. E. Morgan
Storrs, Connecticut

Mark Keeney
College Park, Maryland

E. P. Reineke
East Lansing, Michigan

Evert Wallenfeldt
Madison, Wisconsin

H. D. Eaton
Storrs, Connecticut

Robert Jenness
St. Paul, Minnesota

F. A. Kummerow
Urbana, Illinois

L. H. Schultz
Madison, Wisconsin

G. M. Ward
Manhattan, Kansas

R. E. Ely
Reno, Nevada

C. K. Johns
Ottawa, Canada

A. E. Freeman
Ames, Iowa

M. L. Speck
Raleigh, N. C.

William Hansel
Ithaca, New York

O. W. Kaufmann
East Lansing, Michigan

Durward Olds
Lexington, Ky.

R. W. Touchberry
Urbana, Illinois

C. A. Zittle
Philadelphia, Pa.

COMMITTEE ON JOURNAL MANAGEMENT

B. H. Webb, Chairman
Eastern Utilization Research and Development Division, Washington, D. C.

H. A. Herman
E. O. Herreid, ex officio
J. E. Legates
H. F. Judkins, ex officio

OFFICERS OF THE ASSOCIATION

S. T. Coulter, President
Department of Dairy Industries
University of Minnesota, St. Paul

H. F. Judkins, Sec.-Treas.
32 Ridgeway Circle, White Plains, N. Y.

G. H. Wise, Vice-President
Department of Animal Science
North Carolina State College, Raleigh, N. C.

E. O. Herreid, Editor-in-Chief
Champaign, Ill.

DIRECTORS

Raymond Albrectsen
Ithaca, N. Y.

Stuart Patton
University Park, Pa.

R. E. Erb
Lafayette, Ind.

W. M. Roberts
Raleigh, N. C.

N. L. Jacobson
Ames, Iowa

C. W. Nibler
Lincoln, Nebr.

I. W. Rupel
College Station, Texas

H. F. Judkins, ex officio

E. O. Herreid, ex officio

General Advertising

Space	1 Time	6 Times	12 Times
One Page	$150.00	$132.00	$108.00
Half Page	99.00	87.00	72.00
Quarter Page	52.20	46.20	39.00

Advertising plates, copy, insertion orders, contracts, and requests for information relating to advertising in the Journal should be addressed to the Journal of Dairy Science in care of The Garrard Press, 510–524 North Hickory Street, Champaign, Illinois.

Nonmember Subscriptions

Nonmember subscriptions are $20.00 per volume in the United States and Canada; $20.50 in all other countries, net and postpaid. New subscriptions and renewals begin with the first issue of the current volume. Renewals should be sent in by December 1 of the current year for the following calendar year to avoid a break in the series. Subscriptions should be sent to Claude Cruse, The Garrard Press, 510–524 North Hickory Street, Champaign, Illinois.

Subscriptions for the British Isles and the British Empire should be ordered through our agents: Messrs. Baillière, Tindal and Cox, 7 and 8 Henrietta Street, Covent Garden, London, W.C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert Street, St. Leonards, N.S.W., Australia.

Changes of mailing addresses, post office notices of undeliverable copies, inquiries about copies lost in the mail and all other matters pertaining to the mailing list for nonmember subscribers should be sent to The Journal of Dairy Science in care of The Garrard Press at the above address. Claims for copies of the Journal of Dairy Science lost in the mails must be received within 30 days (90 days foreign) of the date of issue. Notice of change of address must be received two weeks before date of issue.

Memberships

Membership dues in the Association are $12.50 a year and should be sent to H. F. Judkins, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, New York. Renewal should be made not later than December 1 each year.

Changes of mailing addresses, inquiries about copies lost in the mail, requests of back copies and all other matters pertaining to the mailing list for members of the American Dairy Science Association should be sent to Dr. Judkins at the above address. Claims for copies of the Journal of Dairy Science lost in the mails must be received within 30 days (0 days foreign) of the date of issue. Notice of change of address must be received two weeks before date of issue.

Form 3579 to be returned to Garrard Press

Journal of Dairy Science is published monthly by the American Dairy Science Association and printed by
The Garrard Press, 510–524 North Hickory Street, Champaign, Illinois

Second class postage paid at Champaign, Illinois

Copyright, 1963, by the American Dairy Science Association. Printed in the U. S. A.

359734

INSTRUCTIONS TO CONTRIBUTORS

The Style Manual for Biological Journals[1] has been adopted as the guide for authors.[2] Publication of papers is limited to members of A.D.S.A., but only one author of a joint paper need be a member. Those of outstanding merit may be accepted from nonmembers. Papers when accepted become the copyright of the JOURNAL and can be reprinted only by the Garrard Press. Reproduction of graphs, tables, and illustrations for books and other periodicals may be authorized by the Editor-in-Chief.

To be eligible for publication in the JOURNAL, the data in papers must not have appeared previously in any other publication except proceedings of annual or biennial conferences or in symposia.

ORGANIZATION OF PAPERS

1. Title should appear at the top of the first page, be as brief as possible, and be indicative of the research, followed by the author(s) name(s) and affiliation(s).
2. Summary and its preparation.
   a. The summary aids in the dissemination of scientific information, reduces the work and cost of abstracting journals, and is convenient for readers.
   b. The summary must be on the first page, be brief, specific, and factual. It should not exceed 200 words.
   c. The opening sentence should state the research objectives, but the title *should not be repeated.*
   d. It should be intelligible without reference to the original paper and contain complete sentences and standard terminologies. It should be assumed that the reader has some knowledge of the subject.
   e. The author(s) should emphasize newly discovered facts and observations, unique apparatus and techniques, numerical data with statistics, physical-chemical constants, and new methods and their accuracy.
   f. Except in most unusual cases, references to earlier work should be omitted.
3. Statement of the problem and pertinent investigations.
4. Objectives or reasons for research.
5. Experimental procedures.
6. Results.
7. Discussion. (6 and 7 may be combined.)
8. Conclusions are optional.
9. Acknowledgments.
10. References. The minimum of only pertinent references should be used. They must have title(s), author(s) name(s), name of periodical, volume, page number, and year of publication. If a book, publisher's name and address must be added.
11. Manuscripts must be typed double-spaced on 8½- by 11-inch bond paper. Multilithing on bond paper is acceptable. Lines on each page should be numbered from 1 to 26 or 28, to make it easier for the Editorial Board to review papers. The side margins should be one inch wide. Clipped-to, pasted-on, and written insertions are not acceptable. *Pages should not be stapled together.*
12. Figures (graphs) should be made with black India ink on white drawing paper, tracing paper, or blue linen and the sheets should not exceed 8½ by 11 inches. Graph papers with yellow, green, and red lines should not be used, because the lines cannot be filtered out. Curves should be identified with the symbols ○, ⊙, ●, □, ■, △, ▲, ▼, +, or ×, and they should be about 0.8 mm thick, for the axes about 0.5 mm thick, and for grid lines about 0.4 mm thick. Grid lines are necessary only if readings are to be made from the curves. Letters on the abscissae, ordinate, and the figure should be in upper case and be about 3 by 5 mm and about 0.5 mm thick, to be readable when graphs are reduced to column width. Titles for figures (graphs) must be on separate sheets. Following is a well-made figure reduced to size of the printed page:

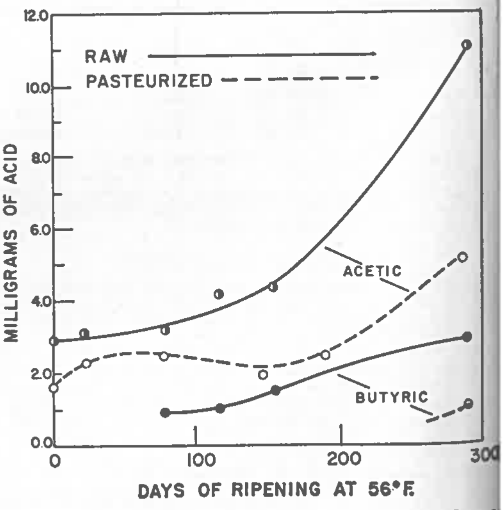


FIG. 1. Acetic and butyric acids in raw and pasteurized milk Cheddar cheese during ripening (milligrams in distillate obtained from 150 g of cheese oil).

[1] American Institute for Biological Sciences, 2000 P Street, N. W., Washington, D. C. Price $3.

[2] J. Dairy Sci., 44: 1788. 1961.

13. Tables should be numbered on the center of the page with the title immediately below, and each table should be typed on a separate sheet of 8½- by 11-inch bond paper. They should be placed together at the end of the manuscript.

Titles should indicate the content of tables and facilitate comparisons, show relationships clearly, be self-explanatory, and save space. Label heading and subheadings accurately and concisely with the data centered under them. Use correct abbreviated dimensions. Data should be referred to and discussed but not repeated in the text, and they should be presented in only significant digits within the accuracy of the methods. Use the metric system whenever possible. Do not use vertical lines and only a minimum of horizontal ones.

Tables are costly. Use graphs whenever possible.

14. Colored illustrations can be reproduced. Authors should submit detailed specifications to the Editor and costs will be supplied.
15. Abbreviations for titles of periodicals and for botanical, chemical, physical, mathematical, and statistical terms should conform to those in the Style Manual for Biological Journals.
16. Terms such as Cottage cheese, Cheddar cheese, Limburger cheese, etc., should be capitalized as indicated. Butteroil, skimmilk, buttermilk, etc., should be written as one word. Milk fat has replaced butterfat.
17. Critical reading of papers, before they are submitted, by persons other than the author(s) will help to clarify statements and eliminate errors.
18. All manuscripts must be submitted to the Editor-in-Chief in duplicate. The ribbon copy, only, of revised manuscripts should be resubmitted.
19. Receipts of manuscripts will be acknowledged. Authors will be notified within 30 to 60 days of the action taken by the Editorial Staff.

TECHNICAL NOTES

20. The technical note section of the JOURNAL is for the publication of research with narrow boundaries, announcement of new findings that may help other scientists, invention of research and teaching devices, establishment of priorities, and for useful information from projects that have been terminated before completion. *This section is not a repository for inferior research.* If the scope of the technical note permits, it may be organized the same as a research paper, except there is no summary at the beginning and the author(s) name(s) and affiliation(s) appear at the end. Otherwise organization is flexible.

Technical notes may or may not be reviewed by the Editorial Board.

OUR INDUSTRY TODAY

21. This section of the JOURNAL is for papers of timely industry interest. If the papers contain applied research, they should be organized the same as research papers, although a summary at the beginning is not required. Regardless of the material in these papers, they should be organized in logical topics.

Papers in this section may or may not be reviewed by the Editorial Board.

PUBLICATION OF EVENTS

22. Material for the announcement of meetings, short courses, and other special events of interest to the Association membership, must be received by the first day of the month and at least two full calendar months before the event is scheduled to take place. For example, the material for any event in February must be received by December 1. These statements do not apply to material for the Annual Meeting.

PEOPLE AND EVENTS

Dean Harold Macy Retires At Minnesota

Harold Macy

DEAN HAROLD MACY, a distinguished microbiologist at the University of Minnesota, retired July 1. He has been a member of the Minnesota staff since 1919, advancing through all academic ranks to full professor, to associate director and then director of the Agridirector of the Agricultural Experiment Station, and finally to Dean of the Institute of Agriculture in 1953.

A native of Hudson, New York, he was awarded the B.S. degree by Cornell University in 1917 and the Ph.D. degree by Iowa State College in 1929. Before coming to Minnesota, he served as bacteriologist for Geneva, New York, and bacteriologist and chief sanitary inspector for the Red Cross. Dean Macy is best known for his investigations in dairy bacteriology. His research on the microorganisms which cause discoloration and off-flavors in butter was fundamental to the solution of mold-infected butter, a defect which caused great losses to the butter factories in Minnesota and the nation. He is the author of 150 papers published in scientific journals, experiment station bulletins, and in industry publications. His fundamental publications on the microbiology of butter are classics and have stood the test of time. He is the co-author of the book Milk and Milk Products, with C. H. Eckles and W. B. Combs. He also compiled two indices for the JOURNAL OF DAIRY SCIENCE.

In addition to his academic and administrative career at the University of Minnesota, Dean Macy served in World War I for nearly 1 yr and served 2 yr during World War II in the Sanitary Corps, mostly in Europe. He was decorated with the Armed Forces Reserve Medal, also with the Chevalier, Order of Public Health and the Chevalier, Legion of Honor, received in France in 1945. In 1955 he was placed on the Army of the United States retired list with the rank of Colonel.

Dean Macy has been granted three leaves to serve abroad. From January to April, 1952, he participated in a survey of agricultural research institutions in Latin America, sponsored by the Technical Cooperation Administration. He was a member of a commission to the Seoul National University of Korea for
the Foreign Operations Administration durin
February 20 to March 16, 1954. From Marcl
8 to May 31, 1956, he served as consultan
for the International Cooperation Administra
tion with the Office of Economic Coordinato
United Nations Command in Korea.

Dean Macy has held offices in a number o
honorary and professional organizations. H
was chairman of the dairy products section o
the American Dairy Science Association i
1934 and a member of the Association's exe
utive board from 1938-39; president of th
North Central Branch of the Society of Ame
ican Bacteriologists, 1939-40; president of th
Minnesota Chapter of Gamma Sigma Delt
1942; and historian and executive committe
member, 1960-61; and president of the Minn
sota Chapter of Sigma Xi in 1942. He wa
elected an honorary member of the Minneso
Veterinary Medical Society in 1958 and
Phi Zeta in 1959.

Other offices and committee chairmanshi
associated with his administrative work
the University of Minnesota include: Chairma
of the advisory committee, Mayo Forestry an
Horticultural Institute, 1947-48; Hormel I
stitute Board as executive secretary and tec
nical adviser, 1949-53, and member since 195
vice-chairman of the State Soil Conservati
Committee, since 1953; and president of t
Dairy Council of the Twin Cities, a unit
the National Dairy Council, since 1956.

In the Association of Land Grant Colleg
Dean Macy has served in several responsib
positions, including: Membership on the exe
utive committee, 1953-55, and the Committ
of Nine, 1953-54; member of the executi
committee, Division of Agriculture, 1953-
and chairman in 1954; member of the Sena
1953-55. Since 1954, he has been a memb
and in 1958-59 was chairman of the Adviso
Board, National Agricultural Extension Cent
for Advanced Study.

Dean Macy has also held important positio
in the organization of Agricultural Expe
ment Station Directors of the North Cent
Region, serving as vice-chairman in 1947-
and secretary in 1949-50. He has also serv
in many advisory and consultant capaciti

His most recent honor was an award
January, 1961, by the Minnesota chapter
the Soil Conservation Society of America
distinguished service to conservation by
vancing the art and science of good land u

For his 44 yr as teacher, researcher,
ministrator, and national and internatio
consultant, Dean Harold Macy has earne
place among our best agricultural statesm

ion during
om March
consultant
dministra-
oordinator,
.

number of
ations. He
section of
ociation in
tion's exec-
ent of the
y of Amer-
dent of the
gma Delta,
committee
the Minne-
2. He was
Minnesota
958 and of

airmanships
ve work at
: Chairman
orestry and
Hormel In-
ry and tech-
since 1953;
Conservation
dent of the
s, a unit of
1956.

ant Colleges
l responsible
on the exec-
e Committee
he executive
ure, 1953-55
f the Senate
n a member
the Advisory
ension Center

tant position
ural Experi-
North Centr
n in 1947-4
s also serve
t capacities.
an award i
ta chapter o
America fo
vation by ad
good land us
esearcher, a
internation
has earned
ral statesme

Maybe nowhere. But that's part of our basic assignment at Groton—trying to improve livestock and poultry production by fitting new-found solutions to age-old problems.

The basic research in the Pfizer Research Laboratory at Groton covers many fields. But these scientists with questioning minds are always looking for possible applications in what seems to be entirely unrelated areas.

That's why you'll see our agricultural specialists keeping close tabs on all the work at Groton—whether it's research on cancer, cardiac problems, or cortico-steroids . . . in the search for better production tools.

This continuous sifting of ideas makes sure that nothing important gets left on the shelf.

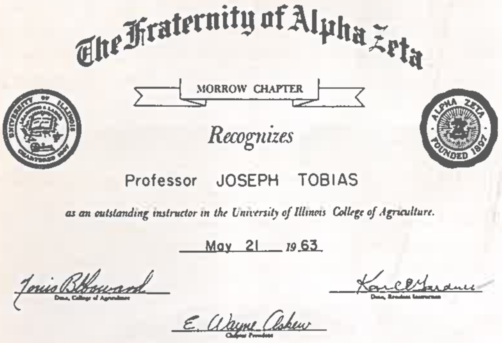
And it's why there'll be better things for livestock and poultry out of Groton in the future.

Pfizer

Chas. Pfizer & Co., Inc.
New York 17, N. Y.
Science for the world's well-being®

Dr. Joseph Tobias Honored At Illinois

At the Annual Recognition Banquet for the College of Agriculture, on May 21, Dr. Joseph Tobias was cited as an outstanding instructor. He was presented with the following certificate:



The Fraternity of Alpha Zeta

MORROW CHAPTER

Recognizes

Professor JOSEPH TOBIAS

as an outstanding instructor in the University of Illinois College of Agriculture.

May 21 1963

Louis B. Howard
Dean, College of Agriculture

Karl E. Gardner
Dean, Resident Instruction

E. Wayne Askew
Chapter President

Born in Czechoslovakia, Dr. Tobias came to this country in 1939. He attended West Georgia College. Later he enrolled in the University of Georgia, where the B.S. degree was conferred in 1942 with a major in Dairy Husbandry.

Joseph Tobias

He served in the U.S. Army from 1942 to 1946, being commissioned a First Lieutenant in 1943, and served in the Midwest Pacific area. He is now a Lieutenant Colonel in the U. S. Army Reserve.

Dr. Tobias earned the M.S. degree in 1949 and the Ph.D degree in 1952, both from the University of Illinois. His major areas were dairy technology and chemistry.

He was appointed to the staff in dairy technology in 1952, with responsibilities for teaching and research. His present rank is Associate Professor of Dairy Technology.

Dr. Tobias' ability as a teacher is evident in his accomplishments as coach of the dairy products judging teams at the University of Illinois. His teams have won four of the past five national intercollegiate contests. This record is unsurpassed in all the history of intercollegiate competition. His classroom lectures and laboratory exercises in applied chemistry exemplify a high order of competence and thoroughness in presenting basic concepts.

Dr. Tobias was selected for the outstanding instructor award by active members of the Fraternity of Alpha Zeta. The great majority of active members of this fraternity are majors in the animal and plant sciences, in agricultural education, in agricultural economics, and in agricultural engineering. This year only t active members are majoring in dairy tec nology.

Dr. Tobias is a member of Alpha Ze Gamma Sigma Delta, Sigma Xi, Phi Kap Phi, and Gamma Alpha. In addition, he faculty adviser to the Agricultural Counc and to Alpha Zeta. He is also a member the American Dairy Science Association a of its dairy products judging committee.

E. B. Carter Retires

E. B. Carter, one of Illinois' leading dai plant operators, has announced his retireme as manager of Beatrice Foods Company's di sion in Decatur, Ill., He will be succeeded his son, R. H. Carter.

E. B. Carter

R. H. Carter

A veteran of 48 yr in the dairy industr E. B. Carter began his career in Bloomingto Illinois, in 1915, and and had been manag of the company's Decatur operations sin 1925.

Under his management, the Decatur pla developed into an outstanding milk operatio It was one of the first dairies in the state homogenize milk and the first to process a sell Half and Half. Its laboratory was t second in the state to be approved by the Sta Health Department for testing milk produc

Mr. Carter also was active in both indust and civic affairs and served as president the Illinois Dairy Products Association a as president of the Illinois Dairy Technolo Society.

Assistant manager and sales manager the Decatur plant since 1960, Richard Cart joined Beatrice Foods in May, 1942, as a me ber of the production staff after attendi the University of Illinois, where he major in dairy technology.

He advanced progressively through a numb of positions and was named plant superinte ent in 1955.

ar only two
dairy tech-

Alpha Zeta,
Phi Kappa
lition, he is
ral Council,
member of
ociation and
mittee.

eading dairy
s retirement
ipany's divi-
succeeded by

I. Carter

iry industry,
Bloomington,
een manager
ations since

ecatur plant
lk operation
the state to
process and
ory was the
by the State
ilk products
oth industry
president of
ociation and
y Technology

manager of
chard Carter
12, as a mem-
er attending
he majored

igh a number
superintend-

---

* * * * * * *
Milestones in Nutrition

1915
when
B vitamins were
missing...

. . . they turned to milk. In 1915 McCollum and Davis found that there was a water-soluble growth factor present in dried milk and wheat embryo. They called it "vitamin B."

Today "vitamin B" has grown into a family of vitamins: thiamin . . . riboflavin . . . niacin . . . pantothenic acid . . . vitamin B_6 . . . biotin . . . choline . . . folic acid . . . and vitamin B_{12}. Some members of the family . . . thiamin and niacin . . . have been found to prevent major diseases of man . . . beri-beri and pellagra. Others . . . folic acid and vitamin B_{12} . . . are effective against diseases of the blood . . . pernicious anemia and sprue.

No one food is rich in all the B vitamins. These nutrients are best obtained by consuming recommended amounts of the four main food groups . . . dairy foods, meats and eggs, vegetables and fruits, bread and cereals. Milk and dairy products . . . 3 to 4 glasses for children and 2 glasses for adults . . . make a balanced contribution to the supply of the B complex vitamins.

As they have in the past, milk and other dairy foods will continue to play a vital role in the nutritional welfare of our people.

The nutritional statements made in this advertisement have been reviewed by the Council on Foods and Nutrition of the American Medical Association and found consistent with current authoritative medical opinion.

Since 1915 . . . promoting better health through nutrition research and education
NATIONAL DAIRY COUNCIL
a non-profit organization
111 NORTH CANAL STREET • CHICAGO 6, ILLINOIS

AVAILABLE ON REQUEST: Reprints of this series of messages on "MILESTONES IN NUTRITION" and "NUTRITION HANDBOOK FOR FAMILY FOOD COUNSELING" booklet

Dr. John J. Sullivan Joins American Breeders Service

DR. J. J. SULLIVAN recently joined American Breeders Service, Inc. as Research Assistant, at Madison, Wisconsin.

Dr. Sullivan received his Doctor of Philosophy Degree from Rutgers University on June 5, where Dr. John P. Mixner was his major advisor. On June 8, he married Miss Joan Lackovich of Cliffside Park, New Jersey. Master of Science Degree was obtained from the University of Tennessee in 1959, for work in the fields of Dairy Science and Zoology, under DR. ERIC SWANSON.

J. J. Sullivan

Dr. Sullivan is a native of Clinton, New Jersey. He grew up on a dairy farm and was active in 4-H Club work for 10 yr, showed registered Jersey cattle, and entered state and national 4-H Dairy Judging contests. He continued these activities as an undergraduate at Rutgers. He served as secretary, vice-president, and president of the Rutgers Dairy Club, and other campus leadership activities. Membership in American Dairy Science Association dates from this period.

His work in the biological research area will be with DR. F. I. ELLIOTT, Stud Manager and Director of ABS Research. With his dairy background, training, and splendid accomplishments as a research worker, he will be afforded a real opportunity to contribute much in his chosen field.

O. H. Kahrl Promoted by Beatrice Foods Company

ORVILLE H. KAHRL has been promoted general engineer for Beatrice Foods Co. veteran of 24 yr with Beatrice Foods, Ka has been assistant engineer for the firm si 1948. He succeeds GORDON HOBBS, gene engineer for the firm since 1937, who reti after 37 yr with the company.

O. H. Kahrl

Gordon Hobbs

Mr. Kahrl started with Beatrice Foods 1939 as a refrigeration service mechanic the Danville, Illinois, ice cream plant. advanced through various maintenance a engineering positions, then was promoted plant superintendent for the Kankakee, I nois, ice cream plant.

Mr. Hobbs began his career with the co pany in the Baxter Springs, Kansas, plant 1926. He was named engineer for the Tope plant and was transferred to the Lincoln, braska, plant as chief engineer in 1931. 1933, he was promoted to district engin for South Dakota, Nebraska, Kansas, Oklahoma plants.

STUDENT NEWS SECTION

W. W. SNYDER, Editor

A Section Devoted to News of Student Members

SPOTLIGHT

The two affiliate chapters of the University of Illinois are featured this month. Recently, the officers of both clubs have found a way to merge some of their activities. We present both reports, with the hope that others may benefit from their activities.

Dairy Technology Society University of Illinois

Previous to 1925, one student club in the Department of Dairy Husbandry at the University of Illinois served the needs of students interested in dairying. Howe when the staff occupied the new Dairy Ma factures Building in the fall of 1925, t move provided the impetus to form a separ group, known as the Dairy Manufactu Club, for students whose main interest Dairy Manufactures. This group has b an active organization since that time, exc for the World War II years. For about yr, Professor P. H. Tracy was the Facu Adviser. Since 1946, Professor S. L. Tuc has been the official adviser. In 1946 the na rice Foods

promoted to
oods Co. A
'oods, Kahrl
ne firm since
BBS, general
who retired

ion Hobbs

ce Foods in
mechanic at
plant. He
tenance and
promoted to
nkakee, Illi-

ith the com-
sas, plant in
r the Topeka
Lincoln, Ne-
in 1931. In
ict engineer
Kansas, and

needs of all
. However,
Dairy Manu-
f 1925, that
m a separate
Manufacture
interest was
up has been
time, except
for about 20
the Faculty
S. L. Tuckey
946 the name

EXPERIENCE COUNTS!

Experience, the universal mother of sciences, the maker of sages, the foodstuff of wisdom, has proven the Sealking plastic-coated paper milk carton.

Over a decade ago, this milk carton brought new milk drinking enjoyment and a greater measure of milk protection and sanitation to millions of American homes.

To dairy processors it offers greater equipment efficiency and plant economy plus an entirely new approach to milk merchandising. To the consumer it offers the only flame-sterilized milk carton with the aluminum seal pouring protection.

Sealright We use SEALRIGHT SANITARY SERVICE for Your Protection **PACKAGEERS TO THE DAIRY INDUSTRY FOR THREE QUARTERS OF A CENTURY**

Sealright-Oswego Falls Corp., Fulton, N. Y., Kansas City, Kansas — Sealright Pacific Ltd., Los Angeles, California — Sealright Canada Ltd., Peterborough, Ontario, Canada.

of the organization was changed to The Dairy Technology Society, and in 1958 it became a student branch of the American Dairy Science Association.

MEMBERSHIP

All students enrolled in the Dairy Technology Curriculum are members without payment of fees or dues. There are 22 members. Officers are elected for 1 yr and consist of a President, Vice-President, Secretary, Treasurer, and Ag Council representative. The Agricultural Council is the central coordinating agency for the 16 specialized student clubs within the College of Agriculture.

SOCIETY ACTIVITIES

Meetings are held at monthly intervals with a speaker, either from a commercial dairy organization or from the faculty of the College. Recently, Professors R. T. Milner and E. O. Herreid showed slides and told of their European trips.

Each fall at Homecoming, the undergraduate and graduate students prepare a luncheon, which is served in the Dairy Manufactures Building, for Dairy Technology Alumni and their families who return for the Homecoming football game. This event provides the main source of annual income for the Society.

THE COMPLETE CULTURE SERVICE
SPECIALISTS IN CULTURE MANUFACTURE FOR 40 YEARS

All lyophylized cultures available in Flav-O-Lac Flakes, "Flakes 40" and Bulk Pkgs.

Standard package Flav-O-Lac Flakes makes 1 quart starter. "Flakes 40" bottle makes 40 quarts *culture* on first propagation . . . no additional incubation, no special treatment involved.

Buttermilk #1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14, 15
Cheese #1, 2, 3, 4, 5, 12, 13, 14, 15, 16, 17, 18, 19
Butter #1, 2, 3, 4, 5
Sour Cream #1, 2, 3, 4, 5

Each of above is a *different blend* of phage-typed strains selected for acid production, flavor/body development in specific products.

Also: S. diacetilactis, S. thermophilus, S. durans (S.D.A.), S. faecalis (D-K), L. bulgaricus, L. acidophilus, L. helveticus, Propionibacterium shermani, Yoghurt culture (3 organisms), Special Cultures on reserve.

Write for information: Whey phage testing service ■ Simplified ordering plan ■ Blend rotation by mail ■ Roquefort, Camembert molds, various Leuconostoc strains on agar slants ■ New 7th Edition, "Friendly Bacteria in the Dairy Industry," 63 pages, illustrated

DALARE ASSOCIATES

THE DAIRY LABORATORIES
2300 LOCUST STREET,
Philadelphia 3, Pa.

Welcoming the Judging Team from Atlantic City in 1962. Left to right: Dean K. E. Gardner; R. T. Milner, Department Head; George Schaufelberger; Paul Hocking; Tom Cain; Marvin Alwes, alternate; and J. Tobias, Coach.

One of the most rewarding activity experiences of the Society has been the sponsoring of the Dairy Products Judging Team. Under the coaching of Professor J. Tobias, the teams from the University of Illinois have achieved a unique and enviable record of accomplishment—four consecutive championships at the National Dairy Products Judging Contest. As a direct result of the teams' achievements, Scott Seibert, team member in 1960, is completing his M.S. degree at the University of Wisconsin and Patricia Crater, team member in 1961, is beginning her graduate study at Ohio State University. Each spring the Society sponsors a students' Dairy Products Judging Contest similar to the National Contest. In addition to the products judged in the National Contest, chocolate ice cream is also included. Numerous valuable prizes, contributed by commercial firms, are awarded to the winners.

Dairy Tech alumni at Homecoming luncheon.

The Society encourages all of its members to achieve excellence in scholarship and makes an award to each student who receives an all-university average of B or higher for any semester. This award consists of an affiliate membership in the American Dairy Science

How to make a good product better...

Make it with Kraft ingredients

For better ice cream . . . KRAFEN spray-dried sweet whey, an ideal alternate for serum solids; lets more natural flavor through, saves 1¢-2¢ per gallon of mix. KRAGELEEN, quality stabilizer controls moisture content, gives ice cream a firm, creamy body, adds to keeping quality, mixes easily. HI-T STABILIZER, a stabilizer for ice cream and frozen desserts that is all-purpose-usable in all production systems—high temperature, and ultra-high temperature short-time units. KRAFT EMULSIFIER is concentrated for easy handling and economy, controls moisture and overrun, provides smooth, even texture.

For better sherbet . . . SHER-STAY gives sherbet firmer body, smoother texture. Lets natural tang of flavoring through, guards against iciness of heat shock. **Frozen Novelties . . .** KRABYN, an instant stabilizer, soluble in cold water, ideal for hot and cold process. Prevents ice crystals, and bleeding of color, flavor, and sugar. **Soft Ice Cream . . .** KRAGEL, emulsifier-stabilizer, combines two ingredients in one. Controls overrun, assures creamy texture. Permits immediate freezing of mix.

For better variety flavors... MINIATURE MARSHMALLOWS, white and colored, also in whipped and syrup form, add new flavor and variety to ice cream and sherbet.

For better chocolate milk and drinks . . . DARI-LICIOUS stabilized chocolate flavored powders combine quality with economy; made from top grade, fine-ground cocoa, stabilized to keep from settling. STABILIZED CHOCOLATE FLAVOR POWDERS from Kraft are offered in a wide range of types to meet any body and flavor requirement. SWEET-K-MALT, evaporated malt extract flavor for frozen confections and ice cream.

For better sour cream dips ... DIP FLAVORINGS by Kraft add new variety, new selling opportunities to sour cream. Tempting French Onion and tangy Spicy Herb flavors. **Fruit Drinks . . .** FRUIT BASE FLAVORS, orange and grape, offer the fresh, natural flavor of real fruit, as well as new sales opportunities for dairies. Kraft Foods Dairy Service Division, Chicago 90, Illinois.

Kraft care . . . the little touch that makes the big difference KRAFT

Association. The number of affiliate memberships awarded per year since the program was started 5 yr ago has increased from one to nine.

Patricia Crater—an outstanding Dairy Technology scholar.

Patricia Crater was an outstanding Dairy Technology scholar, being elected to Gamma Sigma Delta, the Honor Society of Agriculture, and Phi Kappa Phi, the All-University honorary. She also was the fourth high individual as a member of the Judging Team in 1961.

Dairy Technology Society members also encourage outstanding high school students to consider a career in Dairy Technology at the University of Illinois. In March of 1963, George Schaufelberger, senior, and Fred Clusky, freshman, spoke at a Dairy Technology Career Day sponsored by the Department of Food Technology, at the Huntley, Illinois, High School. George Schaufelberger, second high individual in the 1962 National Judging Contest, received a scholarship from the Southern Illinois Dairy Technology Society, and Fred Clusky received one from the Chicago Dairy Technology Society, to aid in defraying their college expenses.

HONORARY MEMBERSHIPS

The Society recognizes excellence not only among its own members but also in other individuals associated with the Dairy Industry. At the annual banquet in 1962, three individuals were awarded Certificates of Merit and Honorary Memberships in the Dairy Technology Society. Carl Hansen, Vice-President of Beatrice Foods Company, Champaign, Illinois; John Wright, Ambrosia Chocolate Company, Milwaukee, Wisconsin; and Nikolai King, dairy scientist of Melbourne, Australia, were selected because of their contributions to the Dairy Industry.

This year a new precedent was established when Mike Campbell, the president of the Dairy Production Club, was also elected vice-president of the Dairy Technology Society; a combined banquet for the two clubs was held. W. E. Peterson, Professor Emeritus, University of Minnesota, was the speaker. Sam E. Dean, Jr., Chairman of the Board of the Dean Milk Company, Franklin Park, Illinois, was honored by the Society with the Certificate of Merit and Honorary Membership in the organization. The Dean Milk Company employs 22 graduates from the curriculum in Dairy Technology at the University of Illinois. This is a record unequaled by any other dairy company in Illinois.

Certificate of Merit presented to Sam E. Dean, Jr. Left to right: Marvin Alwes, President; Professor Emeritus, W. E. Peterson, speaker; and Sam E. Dean, Jr.

Dairy Production Club

The primary purpose of the Dairy Production Club is to bring together students and faculty members who have dairy production interests, thereby stimulating a greater appreciation of all areas in dairy science. To accomplish this aim, the 40 members of the University of Illinois Dairy Production Club meet once each month during the academic year to plan club activities and to discuss timely dairy topics. Members gain leadership experience through their participation on committees and as officers. The officers are selected annually in January and this year they are as follows: President, Michael Campbell; Vice-President, V. Lynn Laible; Secretary, Nannette Smith; Treasurer, Don Pritchard; Ag Council Representative, Erman Schairer; and Reporter, Dan Bock.

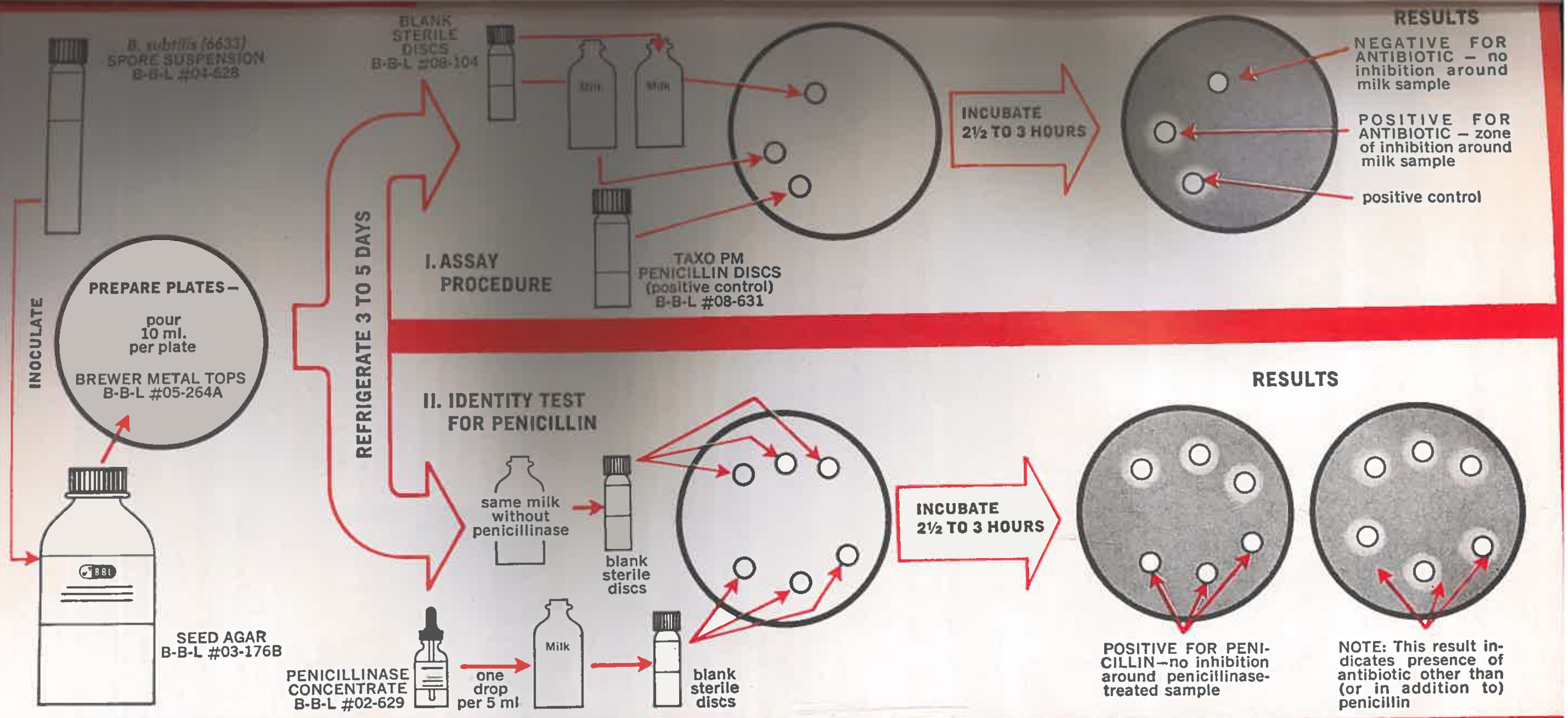
The advisors for the club are selected from the faculty of the Department of Dairy Science. The advisors serve 3-yr rotational terms with one new advisor selected annually. This provides new stimulus and advice for the club. Since 1947, when G. W. Salisbury became Head of the Department, the following men have served various terms as advisors: R. E. Brown, K. E. Gardner, and E. E. Ormiston. The present advisors are J. L. Albright, J. G. Cash, and J. C. DeFries.

am E. Dean,
esident; Pro-
peaker; and

aled by any

iry Produc-
udents and
production
eater appre-
nce. To ac-
of the Uni-
uction Club
ie academic
to discuss
leadership
ion on com-
ers are se-
is year they
l Campbell;
Secretary,
Pritchard;
n Schairer;

elected from
Dairy Sci-
tional terms
ually. This
ice for the
alisbury be-
ie following
is advisors:
E. E. Ormis-
L. Albright,



The presence of antibiotics in milk following mastitis therapy in cows has created serious public health problems and caused technical difficulties within the dairy industry. A rapid, practical laboratory procedure to assist regulatory agencies and the dairy industry in solving these problems was described by Arret and Kirshbaum.* This procedure employs rapid growth of a sensitive strain of *B. subtilis* for assaying the presence of antibiotics in milk and for determining its identity with penicillin. Inhibition of growth by the presence of as little as 0.05 unit of penicillin per ml. of milk sample is detectable within 2½ hours.

In answer to many requests for information about the availability of B-B-L products for this simplified procedure, the B-B-L Development Laboratory has prepared this TECHNICHART. It graphically illustrates the basic procedure, showing the materials necessary—**all of which are available from B-B-L.** A complete brochure with detailed technique and product listing is available upon request. No. 13

*Arret, B., and Kirshbaum, A.: J. Milk and Food Technol. 22:329, 1959.

BALTIMORE BIOLOGICAL LABORATORY, INC.
BALTIMORE 18, MARYLAND
A Division of Becton, Dickinson and Company

B·B·L

B-B-L AND TAXO ARE TRADEMARKS. 81560

The University of Illinois Dairy Production Club and their advisors. The Club meets in the Dairy Science Seminar room in the Animal Sciences Laboratory.

The club programs feature speakers from the University staff and guest speakers who discuss a wide variety of subjects of interest to students in dairy science. For example, speakers this year have been as follows: J. L. Albright, En route to the International Dairy Congress in Copenhagen; L. R. Fryman, Efficient Milking Practices; K. E. Harshbarger, Governmental Nutrition Survey of Southeast Asia; R. L. Hays, Udder Edema; and B. L. Larson, Radioactive Fallout and the Dairy Industry.

This year two different dairy judging teams represented the University of Illinois at the National and International Contests at Waterloo, Iowa, and Chicago, Illinois. Pictured above is the team which placed first in the Holstein breed and fourth overall (out of 31 teams) in the National Intercollegiate Dairy Judging Contest at Waterloo. Left to right are J. L. Albright, coach, and team members V. Lynn Laible, John Duis, Ray Ropp, and Jeff Gillespie.

This year's activities began with a picnic especially planned so that the freshmen could become acquainted with the members, officers, staff, and club program. The members of the dairy cattle judging team were announced by the coach; the candidates had just completed a training trip en route to the Regional Dairy Judging Contest at the Kansas State Fair, Hutchinson, Kansas, where they placed second. After the picnic the judging team left for a practice session en route to the National Contest at Waterloo, Iowa.

Where there are cattle judges, there are showmen. Another fall activity was a showmanship contest sponsored jointly for the first time by the Hoof and Horn Club and the Dairy Production Club. The showmanship contest consists of experienced and inexperienced divisions and an over-all winner was selected. Another event of the evening was a cow-milking contest. Appropriate prizes donated by national dairy organizations were presented at the All-Ag Banquet to the winners of the experienced and inexperienced divisions of the dairy showmanship contest and the spring judging contest.

The Illinois Purebred Dairy Cattle Association sponsors a calf sale each February, offering outstanding calves for 4-H and FFA members. The Dairy Production Club cooperates by consigning a calf purchased from the University farm. The members groom and train the calf for the sale. They also operate a food stand. The profits from the food stand and calf project, which amounted to $360 this

meets in

d the Dairy

ith a picnic
shmen could
bers, officers,
mbers of the
nnounced by
st completed
gional Dairy
State Fair,
placed sec-
ng team left
the National

s, there are
was a show-
tly for the
rn Club and
showmanship
nd inexperi-
winner was
evening was
priate prizes
izations were
to the win-
perienced di-
contest and

ttle Associa-
oruary, offer-
d FFA mem-
b cooperates
rom the Uni-
m and train
so operate a
e food stand
to $360 this

Suit your taste for color and flavor; there is no more profitable variety of Dutch and natural processed cocoa, liquor and granules than...

PETER'S® • RUNKEL'S® NESTLÉ'S®

THE NESTLE COMPANY, INC.
WHITE PLAINS, N. Y.

year, are used to defray club and judging team expenses.

Several club members are shown with the club's consignment to the 1963 Illinois P.D.C.A. 4-H and F.F.A. sale. Standing left to right are V. Lynn Laible, Randall Ross, Michael Campbell, Larry Muller, Erman Schairer, Jerry Miller; and kneeling left to right are David Schingoethe, Dan Bock, and Ray Ropp.

This year the Dairy Production Club cooperated with the Dairy Technology Society to sponsor the first combined Dairy Banquet. Ray Ropp, the past president of the Dairy Production Club, was the toastmaster at the banquet. Jerry Miller, a former judging team member and past club vice-president, was named the outstanding senior and V. Lynn Laible was presented with the outstanding sophomore award. The speaker for the evening was Dr. W. E. Peterson from the University of Minnesota.

In the last spring an Intracollegiate Judging Contest was sponsored by the club. Prizes for inexperienced and experienced contestants and house teams were given. A house team consists of three individuals from the same campus housing group. Approximately 50 University of Illinois students participated in the contest. In addition, the judging class from Purdue University participates in the contest each year.

The top showmen in the 1962–63 Showmanship Contest were (right to left): Jerry Miller, Ray Ropp, John Duis, Jim Behrens, Fred Cluskey, and Art Neavill.

A tour of outstanding dairy farms and dairy organizations is scheduled annually. The purpose of this trip is to observe progressive dairy farmers and new techniques and facilities connected with the dairy industry.

This year the club reporter, with the assistance of club members, is preparing a brochure to describe the club's purposes and activities. This brochure is designed to inform high school students and college students about the Dairy Production Club and the Department of Dairy Science. The club also receives publicity through radio tapes, TV appear-

HOW TO MAKE THE WORLD'S BEST ICE CREAM

You must use the finest dairy ingredients, the finest flavors, the finest mix processing and freezing equipment, the finest packaging—*And The Finest Stabilizer*—KELCO STABILIZER.

Dariloid XL® *for your white mixes*
Sherbelizer® *for your chocolate mixes*
Products of **KELCO COMPANY**

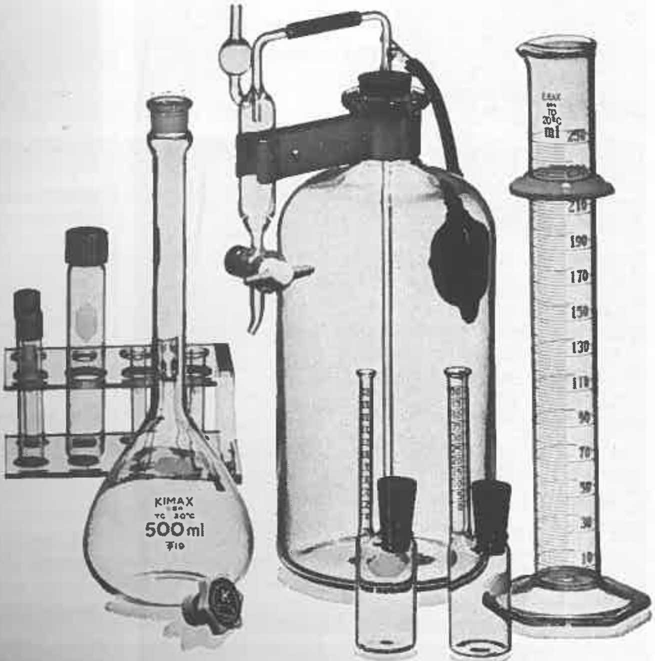
KIMBLE DAIRY TEST GLASSWARE...

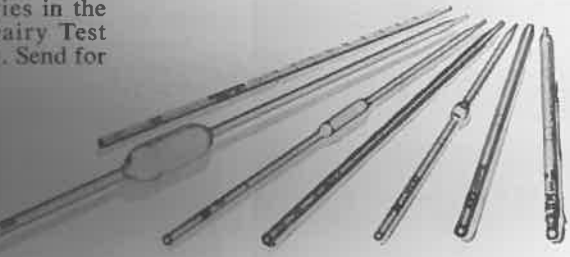
features new utility and economy

The new line of Kimble Dairy Test Glassware includes many important time and money-saving features to ease and speed your testing procedures.

- Many items have been converted to KIMAX* "hard" glass for longer life—greater resistance to chemical attack, heat and mechanical shock.
- Redesigned acid test bottles include TEFLON* stopcock plugs to prevent contamination of test solutions, eliminate freezing and binding of stopcock plugs.
- We've color-coded volumetric, serological and bacteriological pipets by size (at no extra cost) to save you time in selection and sorting.
- Graduated cylinder #20025 now comes equipped with free SAFE-GARD* plastic bumpers. Use them to mark liquid levels and to prevent damage to cylinders.
- SAFE-GARD* closures for #28015-P volumetric flask offer protection from breakage and cost less than conventional stoppers.

You'll find these and many other quality glassware accessories in the new Kimble KIMAX Dairy Test Glassware Catalog (SP-83). Send for your copy today!





TEFLON is a registered trademark of E. I. duPont & Co., Inc. Manufactured under FISCHER & PORTER Patent No. 2,876,985

*Kimble Glass Company trademark

New 26-page catalog showing the latest and best in dairy test glassware.

Kimble Glass Company, Subsidiary of Owens-Illinois,
Toledo 1, Ohio
Send new KIMAX Kimble Dairy Test Glassware Catalog.

Name____________________

Company____________________

Address____________________

City____________Zone____State______

KIMBLE DAIRY TEST WARE
AN (I) PRODUCT

OWENS-ILLINOIS
GENERAL OFFICES • TOLEDO 1, OHIO

STOP!

You can expect people to buy your product . . . once!

But unless it passes their taste and sight approval, what then? What happens to the rest of your output . . . and your company?

That's where the man from National Pectin comes in!

He can help you offer better-tasting, better-looking products . . . products that will move from the dealer's counters because they are better. Products that will maintain that freezer-fresh goodness!

Write, phone, or wire collect the man from National Pectin. Our complete facilities, experimental ice cream plant and laboratory are at your service. No obligation, of course.

COPENA or
COPENA SPECIAL:
stabilizer-emulsifier
for ice cream

COPENA SS:
special purpose
stabilizer-emulsifier
for soft-serve
products

SURE BET and
EXCEL BET:
for sherbets
and ices

National PECTIN PRODUCTS Company

2625 W. Cullerton • Chicago 8, Ill. • Phone: (312) BIshop 7-0070

The University of Illinois dairy judging tea which placed first in the 1963 Hoard's Dairyma Collegiate Judging Contest, topping a field of 1 entries.

ances, and press releases. An exhibit is pr pared about the club by its members for di play at Ag Student Guest Day. As part their public relations program, the club se a delegation of officers and club members the American Dairy Science Association mee ing in June.

Kentucky

Kentucky's dairy industry is cooperati closely with the Department of Dairy Scien University of Kentucky, in the recruitme of students in the field of Dairy Manufactu ing. The industry has set up a system scholarships and is opening its facilities visits by interested students.

Kenneth E. Mennen, Armour Creameri Springfield, Kentucky, is chairman of the Ke tucky Dairy Education Committee and r cently told a group of 22 University of Ke tucky freshmen that the Dairy Departmen at both Kentucky and Purdue have ten jo for every student they have. He called t shortage of trained personnel critical.

The college students were on a field tr to visit several dairy plants and farms the state. This group visited Armour Crea eries, Springfield, Dean Milk Company, Lou ville, Kraft Foods Co. (cheese plant), La renceburg, Kentucky, Artificial Breeding A sociation's stud and a dairy farm. Al Didier, Dean Milk Company, was host to t group for lunch.

The Education Committee is helping in t recruitment program by furnishing schola ships valued at $2,400 this year for studen in dairying. The committee members are al attempting to find suitable candidates amo the young men at the high school level. Th sponsor these prospective dairy majors at t annual Dairy Career Day held by the Unive sity.

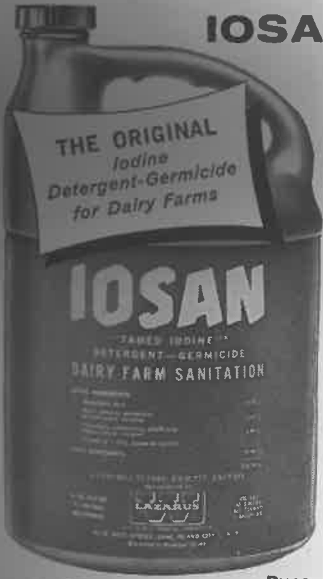
More than 60 high school students, spo sored by 11 dairy companies and three pr ducer groups, attended the recruiting eve this year. The prospective students were i

University of Kentucky freshmen and sophomore students are shown how Cottage cheese is made in the new Dean Milk Company plant in Louisville, Kentucky. The students were on a field trip to visit several dairy plants and farms. Al J. Didier, manager of the plant, explains the process to students John Effinger, Don Schaefer, Babram Goshtosbpour, and Carl Moneyhon. K. E. Mennen, Armour Creameries, Springfield, also watching the process, is chairman of the Kentucky Dairy Education Committee.

troduced in small groups to a faculty member who served as their guide and counselor for the day. After surveying the dairy production facilities, each group toured places of interest on the campus on their way to lunch. After surveying the dairy production facilities, each group toured places of interest on the campus on their way to lunch. Following lunch these high school students heard talks by the President of the University, Frank Dickey, a Carnation Company official Herbert Glenn, and Dr. Stanley Wall, Associate Dean of the College of Agriculture. Dr. D. M. Seath, Head of the Department of Dairy Science, presided at this meeting. Following a tour of the dairy manufacturing laboratories, the boys were treated to all the ice cream they could eat, and later the boys and their sponsors were the University's guests at a baseball game. The evening was spent at the Dairy Club's annual Honor Banquet.

Purdue

Dairy Club has finished a year of rebuilding in 1962-63, with the result that over 50% of all dairy majors and graduate students were enrolled as members. These members favored the club with excellent attendance at the monthly meetings and were privileged to hear several excellent speakers. Dr. Ralph Erb, Assistant Head of the Animal Sciences Department, spoke on the functions and activities of the American Dairy Science Association and the Student Branch of the American Dairy Science Association. Dr. Will Dillon of the Purdue Dairy Extension Service spoke on the opportunities in the dairy industry and



IOSAN® DOES ALL
4 DAIRY JOBS
•Cleans } bulk tanks and all
•Sanitizes } equipment thoroughly
•Removes and prevents milkstone build-up
•Helps control mastitis

LAZARUS
LABORATORIES INC.
Division, West Chemical Products, Inc.,
42-16 West St., Long Island City 1, N. Y.

ALSO MAKERS OF Dyne, Iobac, Pipeline Powdr Plus, Super Powdr Plus, Foamchek, and Foamchek-100

pointed out that over ten inquiries for trained dairy production and dairy manufacturing specialists are received for every graduate. Mr. K. J. Lindsey, Sales Supervisor for the DeLaval Company, spoke on modern milking practices. Mr. Ward Holm, of the Indiana Dairy Products Association, spoke on An American Dairyman in Russia and showed many excellent color slides of his recent trip through Russia. Mr. Cornelius Miracle, Manager of the Normandy Farm, Indianapolis, Indiana, spoke on farm management as a future for dairy production graduates. He also discussed Purdue University's research and management program at Normandy Farm.

At a recent meeting the club members elected the following officers for the next year: Myron Bozel, president; Philip Gordon, vice-president; Andrew Azpell, treasurer, Joyce Smith, secretary; and Philip Stoltz, publicity director.

Club officers and members are making plans and preparations for next year's program, which is to include a membership drive, increased student-faculty functions, several money-making projects, and a service program to the 4-H and FFA clubs of Indiana. The Club, under the continued help and guidance of their advisor, Dr. Dean Frazeur, is looking forward to the next year with much enthusiasm.

Accurate profit-control is the job of Mojonnier Milk Testers for standardizing butterfat and total solids content. • Mojonnier Testers have been a must for profitable dairy operation over 45 years. • Fat Determination accurate to .03 of 1%. Total Solids Tests accurate to 1/10th of 1%.

Write for full details today

MOJONNIER BROS. CO.
4601 W. OHIO ST., CHICAGO 44, ILLINOIS

Mojonnier MILK TESTERS
QUALITY ENGINEERED FOR ECONOMY

STANDARD THROUGHOUT THE WORLD

Tennessee

The annual spring banquet of the Dair
Club of the University of Tennessee serve
as the climax for the year's activities. Dr
H. A. Herman, Executive Secretary of th
National Association of Artificial Breeder
was the main speaker. His topic was oppor
tunities in the field of dairy production.

The student awards were presented by Dr
J. T. Miles, Head of the Dairy Departmen
The Purina Bowl and the Virginia Dare awar
were presented to Gerald Martin, senior i
dairy production and president of the Dair
Club. John Housely, a junior, received tw
awards in the club-sponsored Judging Con
test. He was the winner in the dairy cattl
judging division as well as the over-all con
test winner. Tom Strasser, a junior, was th
winner of the dairy products judging divisio
The ambrosia chocolate award was also pre
sented to Mr. Strasser.

President Gerald Martin presented the Ten
nessee Dairy Science award for 1963 t
Thomas B. Mayfield of Athens, Tennesse
This award is presented annually by the Clu
to a person who has made an outstandin
contribution to the Tennessee Dairy Industr
Mr. Mayfield is President of Mayfield Crean
ery, Athens, Tennessee.

Gerald Martin, Dairy Club president, presentin the 1963 Tennessee Dairy Service Award to M Tom Mayfield, President of Mayfield Creamer Athens, Tennessee.

Ohio State Holds Research Symposium

The Second Dairy Technology Research Sy
posium for Management was held on Th
Ohio State University Campus, May 23. Th
Symposium was designed to acquaint dai
leaders in Ohio with the research program
the Department of Dairy Technology and
attempt to interpret the research findings

of the Dair
nessee serve
ictivities. D
retary of th
cial Breeder
ic was oppo
oduction.

sented by D
y Departmen
ia Dare awa
tin, senior i
of the Dair
received tw
Judging Co
e dairy catt
over-all co
inior, was th
dging divisio
was also pr

ented the Te
for 1963 t
ns, Tenness
ly by the Cl
n outstandi
airy Industr
ayfield Crea

dent, presenti
Award to M
yfield Creame

Symposium

Research Sy
held on T
, May 23. T
acquaint dai
rch program
nology and
rch findings

---

terms of management decisions and industrial needs.

The Symposium included, in brief, 1) personalized tours of the Department's research facilities, featuring displays of major areas of research and research tools and techniques, 2) a luncheon followed by recognition ceremonies, addresses, and a review of the Department's research program, 3) a Seminar address, and 4) a banquet.

Highlight of the day was the luncheon with the first Meyer-Antrim Lecture—a lecture series established to give recognition to H. F. Meyer, President, Meyer's Dairy, Cleveland, and to Everett Antrim, retired dairy industrialist, Worthington, for their roles in encouraging and supporting Dairy Technology research at The Ohio State University. Certificates of Recognition were presented to Meyer and Antrim by Professor Emeritus L. H. Burgwald. Dr. A. B. Garrett, Vice-President in Charge of Research, The Ohio State University, delivered the Lecture entitled The Stake of Commercial Industry in University Research. Dr. W. E. Krauss, Associate Director, Ohio Agricultural Experiment Station, Wooster, concluded the luncheon program with an address—Advancing Knowledge Through Experiment Station Research.

Afternoon presentations included a review of the Department's research by Drs. I. A. Gould, W. L. Slatter, W. J. Harper, and T. Kristoffersen; and a lecture by Dr. E. O. Herreid, Professor of Dairy Technology, University of Illinois—Technical Barriers to Development of Fresh-Flavored Sterile Fluid Milk Products—which was presented before the Inter-Departmental Seminar of the Institute of Nutrition and Food Technology. At the banquet program, presided over by Dr. O. F. Garrett, Director of Research, M & R Dietetic Laboratories, Columbus, Dr. Herreid spoke on the subject, Some Vital Areas of Research on Milk and Its Products.

Participants in Dairy Technology Recognition Luncheon. A. B. Garrett, E. Antrim, H. F. Meyer, W. E. Krauss, and L. H. Burgwald.

Wisconsin

The Babcock Dairy Science Club of the University of Wisconsin highlighted the year's activities with their annual spring banquet.

NOW IT'S OFFICIAL

About American Food Laboratories'

ILS* AND CREAMED COTTAGE CHEESE

INSTANT LECITHINATED STABILIZER TYPE LC-800, a new liquid stabilizer developed exclusively for the stabilization and improvement of creamed cottage cheese is now an optional ingredient of cottage cheese as defined by the new Federal Standards of Identity.

We have long known how *ILS* improves the quality of creamed cottage cheese by making a uniform product, helping the cream adhere to the curd and substantially or entirely reducing wheying-off. *ILS* has been used successfully by many local dairies for the past three years, based on excellent results obtained by the Michigan State University Department of Food Science**.

The properties of *INSTANT LIQUID STABILIZER* prevent balling and lumping of gums and assure complete utilization of all the stabilizer used – ideal for HTST operations. *ILS* also provides the popular mild, sweet cheese with desirable creamy characteristics without the undesirable drainage.

Average rate of use is less than 0.20%. *ILS* is available in pre-weighed unit cans, so that one unit may simply be poured into the pasteurizing vat.

Plant demonstrations with free samples of *ILS* may now be arranged by writing to

★ CARE AND CONTROL

AMERICAN FOOD LABORATORIES, INC.

1000 STANLEY AVENUE, BROOKLYN 8, N.Y.
1442 Flower Street, Glendale 1, California

*ILS is a patented and registered product of AMERICAN FOOD LABORATORIES, INC.

**Original article, published in THE MILK DEALER, available upon request.

The Babcock Dairy Science Club annual spring banquet.

Graduating seniors were honored for their Club activities and scholastic achievements.

The challenges and opportunities in the dairy industry were discussed by three dairy industry leaders: W. C. Johnson of the Wisconsin Section of the American Dairy Association, Harry Palmiter, representing the Wisconsin Cheese Maker's Association, and Bruno Stein of Marschall Dairy Laboratory, Inc. Dr. H. E. Calbert, Chairman of the Department of Dairy and Food Industries, was the main speaker for the banquet.

Theses Completed

M.S. Degree:

RICHARD C. BERNETT. Ad libitum versus controlled feeding of concentrates to lactating dairy cows. Southern Illinois University.

B. K. CHAKRABORTY. Prolonging the kee ing quality of creamed Cottage cheese. T Ohio State University.

ROGER C. HUNTER. Acid degree value a flavor of raw milk as influenced by certa selected factors. University of Wyoming.

D. B. KEKARE. Some milk serum prote responses to heat and to interactions in mi sera. The Ohio State University.

S. P. OHRI. The quality of raw milk selected Ohio markets. The Ohio State Un versity.

Bound Volumes of the Journal For Sale

C. E. POE, 875 16th Street, Boulder, Col rado, has complete volumes 1 to 36 (1917 1953) for sale. They are all bound and excellent condition.

Bind Your Copies of the Journal of Dairy Science

The Alexander Bookbinding Company has made an arrangement whereby members of A.D.S.A. and subscribers to the JOURNAL OF DAIRY SCIENCE can have their copies bound for only $4.95 for six issues and an extra charge of $2.48 for all twelve issues bound together. Most people want their JOURNALS bound in six issues rather than twelve, because of bulk.

We bind with the finest grade buckram, washable hard-bound case, with your name imprinted on the front cover.

Ship journals parcel post. Bound volumes will be returned transportation prepaid. Full remittance must accompany order.

Alexander Bookbinding Co.
SCHOOL–LIBRARY–MEDICAL BINDERS
605 East Eureka
Champaign, Illinois
Telephone 352-6614
Area Code 217

Advertisers' Index

Alexander Bookbinding Co. 2
American Food Laboratories, Inc. 2

Baltimore Biological Laboratories 1

Dairy Laboratories 1
Difco Laboratories ....4th Cov

Kelco Manufacturing Company 1
Kraft Foods 1

Mojonnier Bros. Company 2

National Dairy Council ....
National Pectin Products Company 1
The Nestlé Company, Inc. 1

Owens-Illinois Glass Company
(Kimble Glass) 1

Chas. Pfizer & Company, Inc. ....

Sealright Company, Inc. ....

West Chemical Products 1

University.
ging the kee
ge cheese. T

gree value a
ced by certa
' Wyoming.
serum prote
actions in mi
y.
f raw milk
hio State U

nal For Sale
Boulder, Co
to 36 (1917
bound and

ndex

RESEARCH PAPERS

TURBIDIMETRIC METHOD FOR ESTIMATING SUM OF β- AND κ-CASEINS IN WHOLE CASEIN [1]

H. TESSIER, D. ROSE, AND J. R. MARIER
Division of Applied Biology, National Research Council, Ottawa, Canada

SUMMARY

The amount of $\beta + \kappa$-casein present in whole casein was estimated in a buffered solution of acid casein in the presence of calcium, by means of the difference in optical densities (400 mμ) at 0 and 35 C; the concentration of α_s-casein was obtained by difference. By subtracting the κ-casein content (estimated by sialic acid analysis), the amount of β-casein present in the $\beta + \kappa$-casein mixture was obtained.

Analysis of various test solutions containing known amounts of α_s-, β-, and κ-caseins gave results which agreed within ±2.3%. The method is simple, convenient, and suitable for routine determinations. Analysis of caseins obtained from individual lots of skimmilk indicated that β-casein accounted for 19-36%, κ-casein for 10-23%, and α_s-casein for 49-60% of the total casein.

The only methods presently available for the quantitative estimation of the main fractions of casein are moving boundary electrophoresis and ultracentrifugal sedimentation. These methods are time-consuming and there is a need for a simple test which can be used routinely.

Waugh (13, 14) and McKenzie and Wake (6) have shown that micelles are formed by the interaction of α_s-, β-, and κ-caseins in the presence of calcium, and that the degree of aggregation is influenced by temperature. Waugh reported (14) that solutions of β-casein containing calcium remain clear at temperatures below 18 C, but readily precipitate at higher temperatures. Zittle and Pepper (17), by means of turbidity measurements, studied the influence of various factors (including temperature) on the rate of aggregation of casein, but only over the temperature range 25-35 C. The above observations suggested that a method for estimating a casein fraction in a mixture could be based on the change in turbidity with change in temperature. Development of the method is described herein.

MATERIALS AND METHODS

All caseins were prepared from fresh bulk milk obtained from the Central Experimental Farm, Ottawa. Acid casein was precipitated at pH 4.6 from bulk skimmilk by the addition of hydrochloric acid, then washed several times with water. α-Casein complex (i.e., $\alpha_s + \kappa$-casein) and β-casein were prepared by urea fractionation according to Hipp et al. (3), except that the sodium chloride treatment was omitted during purification of α-casein complex. Each fraction was purified by repeating the procedure three times. Calcium-sensitive (α_s) casein was obtained by treating α-casein complex with 12% trichloracetic acid according to Swaisgood and Brunner (12), and also by the method of Zittle et al. (16) and Morr (9). The procedures were repeated three times to remove traces of κ-casein. κ-Casein was prepared by the method of Swaisgood and Brunner (12), Zittle (15), and Morr (9). The initial preparations of κ-caseins were dissolved in sodium hydroxide at pH 7.0, and residual traces of α_s-casein were precipitated with calcium chloride (0.25 M). All casein preparations were freeze-dried and stored at 4 C.

Moving boundary electrophoresis patterns were obtained on the casein fractions with a Spinco Model H apparatus, 11-ml cells, veronal buffer pH 8.1-8.3, μ 0.1, at 1 C. Estimation of the peak areas of these patterns indicated the following degrees of purity for the casein fractions used: α-casein complex, 97%; α_s-casein, 97%; β-casein, 93%; κ-casein, 93%. Published values for the sialic acid content of α_s- and β-caseins (2), and our own analyses of the preparations used in this work (5), confirm these degrees of purity if it is assumed that the impurity is mostly κ-casein. The estimated purity of the κ-casein preparation agrees with the data of Swaisgood and Brunner (12). The α-casein complex (i.e., $\alpha_s + \kappa$) used contained 18.3% κ-casein by sialic acid analysis.

Received for publication April 4, 1963.

[1] Issued as N.R.C. No. 7483.

RECOMMENDED PROCEDURE

Imidazole-calcium reagent. Dissolve 1.201 g of reagent-grade calcium carbonate in approximately 800 ml of distilled water with 2.0 ml concentrated hydrochloric acid, then add 0.680 g of imidazole, adjust to pH 7.0 with 1 N sodium hydroxide, and make to one liter.

Test solution, prepared from dry casein preparations. Dissolve 0.075 g of dry acid casein or mixed casein fractions in 20 ml of water containing 0.13 ml of 1.0 N sodium hydroxide. After dissolution, adjust to pH 7.0 with 1.0 N hydrochloric acid, and dilute to 25 ml with water. The final casein concentration of this preparation is 0.3%.

Test solution, prepared from skimmilk. Pipette an aliquot of skimmilk containing 0.075 g of casein into a 15-ml centrifuge tube containing 10 ml of water. (Note: The volume of skimmilk required is calculated from casein N determinations; the aliquot required varied between 2.2 and 3.2 ml.) Precipitate the casein by adding 0.125 ml of 1.0 N hydrochloric acid and centrifuge 5 min at 580 × g. Wash the precipitate by suspending twice in 0.1 N sodium acetate buffer (pH 4.6) and once in distilled water, centrifuging as before. The precipitate is then dissolved, adjusted to pH 7.0, and diluted to 25 ml as outlined in the procedure for dry casein preparations.

Turbidimetric measurements. Pipette 5.0 ml of casein solution and 10.0 ml of the imidazole-calcium reagent, both at 0 C, into a cold colorimeter tube, mix thoroughly, equilibrate for 45 min in an ice bath, and measure the turbidity at 400 mμ with an Evelyn colorimeter (1.9-cm light path), using 15 ml of water as a blank. After this first reading, place the samples in a bath thermostated at 35 C for 10 min and again measure the turbidity at 400 mμ. (Note: The samples must be removed from the baths one at a time, wiped dry, and read immediately, so as not to introduce temperature variations.) Determine the difference in optical densities at 35 and 0 C (Δ OD) and calculate the % $\beta + \kappa$-casein by reference to a standard curve prepared with known mixtures of α_s-, β-, and κ-caseins.

RESULTS

In the presence of 0.02 M calcium, neither α_s- nor β-casein could be maintained in suspension at 35 C. κ-Casein gave almost a clear solution at both 35 and 0 C (Δ OD essentially zero). However, solutions of α-complex or whole casein formed reproducible suspensions at either 35 or 0 C, and the turbidity of such suspensions changed markedly with temperature. This effect was produced by the κ-ca present in the α-casein complex, and by b β- and κ-caseins present in whole casein. cause κ- and β-casein appeared to affect turbidity in a similar way, the relation of sum of β- and κ-casein with turbidity studied.

The concentration of casein (0.1% in a fi test mixture) used for the test was selected that both the 0 and 35 C readings were wit the optimum range of the instrument used, to give a maximum optical density differe (Figure 1). At the calcium concentration u

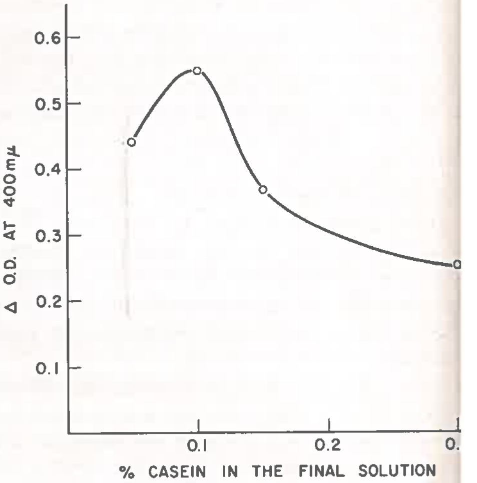


Fig. 1. Effect of acid casein concentratio the final solution on Δ OD at 400 mμ (8 mM/ of calcium throughout).

(8 mM/liter in the final test mixture), ca concentration was quite critical, because hi concentrations gave rise to a coarse suspen with proportionately low OD at 35 C.

The effect of calcium concentration studied over the range from 2 to 14 mM/l in the final test mixture. Turbidity at both and 0 C increased with increasing calcium, the net effect at fixed casein concentration a decrease in Δ OD (Figure 2). When calc ion was reduced to 6 mM/liter or less, plot of Δ OD vs. per cent $\beta + \kappa$-casein did extrapolate to zero Δ OD at zero $\beta + \kappa$-ca This observation suggests that under these ditions insufficient calcium was present to mote normal micelle formation; therefore mM/liter calcium in the final test mixture used. The calcium concentration in this tes critical and should be controlled accurately obtain reproducible results with diffe batches of imidazole-calcium reagent.

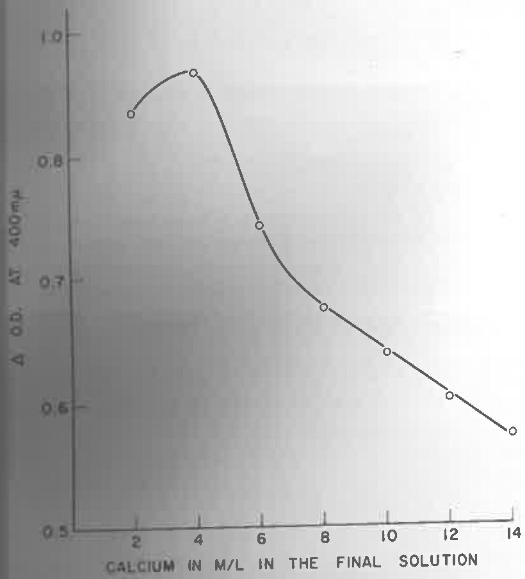


FIG. 2. Effect of calcium concentration in the final solution on the Δ OD at 400 mμ (0.1% whole casein throughout).

Variations in final pH between 6.0 and 8.0 had a negligible effect on the Δ OD. Imidazole buffer does not react with calcium and was, therefore, chosen to maintain a constant pH.

The effect of holding times at 35 and 0 C on optical density after the addition of the imidazole-calcium reagent is shown in Table 1. The turbidity of the solution held at 0 C increased gradually, reaching a maximum after 40 min; results were easily reproducible at 45-60 min. At 35 C, equilibrium was attained within 10 min.

Development of the standard curve. Since standard casein fractions of 100% purity are not available, development of a standard curve relating Δ OD to % β + κ-casein in the mixture involved making allowances for the impurities present in each fraction. To avoid bias in the curve, a large number of mixtures and several preparations of each fraction were used, with corrections applied as follows:

1. α-Casein complex containing 18.3% κ-casein based on sialic acid determination (5)
2. α-Casein complex + β-casein, assuming 18.3% κ-casein in the complex and 7% in the β-casein
3. α_s-Casein + κ-casein, assuming 3% κ-casein in the α_s-casein (the 7% β-casein in the κ-casein does not affect the results)
4. Mixtures of α_s-, β-, and κ-caseins, assuming 3% κ-casein in the α_s-casein, and that the β- and κ-casein preparations were contaminated only with each other.

Data obtained with these mixtures, under the conditions specified above (0.1% casein and 8 mM calcium in the final test solution, pH 7.0) are shown in Figure 3.

With test solutions containing α_s- and κ- (but no β-) caseins, the turbidity differential obtained at 35 and 0 C was directly related to the proportion of κ-casein present in the mixture. Analysis of the α-casein complex, obtained by urea fractionation, indicated a κ-casein content of 18.0%; this value was in good agreement with the value of 18.3% estimated from sialic acid analysis.

Analysis of known proportions of α_s-, β-, and κ-caseins invariably produced differential turbidity measurements related to the sum of β- and κ-caseins present. The relation was the same as that obtained for simple α_s-κ-casein mixtures. From these results it was concluded that the method could be used as a reliable index of the sum of β- and κ-caseins present in whole casein. The proportion of β-casein can be calculated by subtracting the κ-casein content, as estimated by sialic acid analysis (5). The undetermined casein fraction consists largely of α_s-casein.

TABLE 1

Effect of holding time at 0 and 35 C after the addition of imidazole-calcium reagent on the optical density of α-complex + β-casein mixtures

β + κ	Optical density at 400 mμ							
	Time, min, 0 C					Time, min, 35 C		
%	10	20	30	40	60	10	20	30
26.2	0.491	0.549	0.569	0.573	0.577	0.959	0.959	0.959
34.4	0.438	0.491	0.512	0.523	0.530	0.989	0.989	0.989
42.6	0.340	0.392	0.412	0.423	0.426	1.000	1.000	1.000
50.8	0.274	0.321	0.340	0.347	0.349	1.011	1.022	1.011
59.0	0.185	0.218	0.229	0.240	0.242	1.000	1.011	1.022

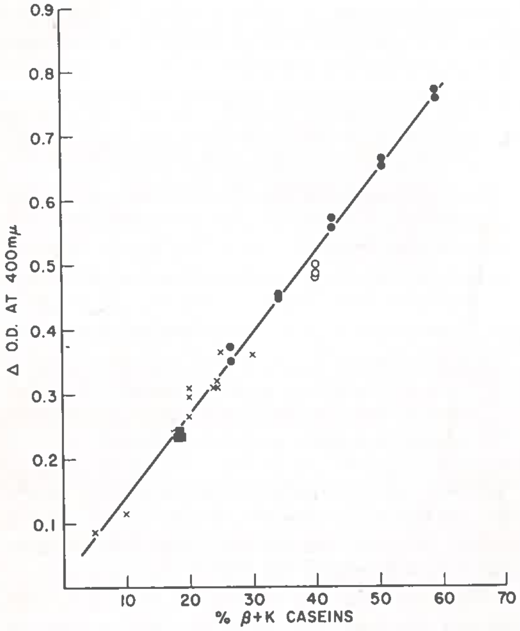


FIG. 3. Calibration curve obtained with various casein mixtures. ■, α-complex; ●, α-complex + β; ×, $\alpha_s + \kappa$; ○, $\alpha_s + \kappa + \beta$.

Statistical analysis of these data indicated good reproducibility, and gave the relation

$$\Delta \text{ OD} = 0.0129 + 0.0128 \pm 0.0004 \ (\% \ \beta + \kappa\text{-casein})$$

or

$$\% \ \beta + \kappa\text{-casein} = (\text{approx}) \ 76 \times \Delta \text{ OD} \pm 2.3\%$$

During development of the standard curve, it was noted that when a mixture of α_s- and κ-caseins, purified by passing three times through a diethylaminoethyl-cellulose column, was used in the test (10), the turbidity at 0 C developed extremely slowly. Readings taken after 1 hr gave values which deviated from the calibration curve by as much as 6%, indicating an inconsistent development of turbidity. No attempt was made to establish a new set of conditions for caseins treated in this manner.

Analysis of caseins. The method was applied to the routine estimation of the proportion of β + κ-casein present in whole acid casein; sialic acid determinations (5) were used to estimate the κ-casein. β-casein and α_s-casein were then determined by subtraction.

Results of routine determinations with individual skimmilk caseins are shown in Table 2. The proportion of β + κ-casein present in whole casein varied between 40.5 and 51.5%, while that of α_s-casein was between 48.5 and 59.5%. β-casein comprised from 19 to 36.3%, and κ-casein from 9.7 to 22.7% of the total casein.

TABLE 2

Analysis of casein fractions of skimmilk

Cow no.	% Casein			
	β + κ	κ [a]	β	α_s-Casein
1	40.5	21.5	19.0	59.5
2	44.5	21.7	22.8	55.5
3	46.0	22.7	23.3	54.0
4	41.5	16.1	25.4	58.5
5	45.7	18.9	26.8	54.3
6	43.5	14.9	28.6	56.5
7	44.5	9.7	34.8	55.5
8	49.3	13.0	36.3	50.7
9	51.5	11.6	39.9	48.5
10	50.4	17.3	33.1	49.6

[a] κ-Casein estimated from sialic acid analy
(5).

DISCUSSION

Originally, this method was expected
measure β-casein only, because this form
casein is monomeric at low temperatures b
readily aggregates at temperatures above 18
(13, 14). However, under the conditions
the test, the aggregation of κ-casein with
casein affected the turbidity in the test in
manner quite similar to that of β-casein wi
the α-casein complex.

Studies with known mixtures of the vario
caseins have shown that some aggregation
curs at 0 C, but that it requires up to 40 m
to attain equilibrium. When heated to 35
a much greater degree of aggregation is ra
idly attained. Increasing the proportions
κ- or β-casein in the mixture decreased t
turbidity of the solution, and this is most a
parent at 0 C.

The possibility that the γ-casein present
whole acid casein behaved in the test similar
to β- and κ-casein cannot be disregarded co
pletely. Hipp et al. (3) indicated a close sim
larity between β- and γ-casein based on th
solubility of these two fractions at 2.5 an
25 C. If this is the case, the values report
in this paper for β-casein would be in err
by the amount of γ-casein present. The valu
reported for γ-casein content of whole ac
casein range from 3-9% (1, 4, 7, 11); howeve
the average value of 3% obtained by Hipp
al. (3) appears to be more reliable for mi
lactation milk.

REFERENCES

(1) BOHREN, H. V., AND WENNER, V. R. N
ural State of Milk Proteins. I. Compo
tion of the Micellar and Soluble Casein
Milk After Ultracentrifugal Sedimentati
J. Dairy Sci., 44: 1213. 1961.

(2) Cayen, M. N., Henneberry, G. O., and Baker, B. E. Studies on Casein. IV. The Sialic Acid Content of Casein. J. Dairy Sci., 45: 706. 1962.

(3) Hipp, N. J., Groves, M. L., Custer, J. H., and McMeekin, T. L. Separation of α-, β-, and γ-Casein. J. Dairy Sci., 35: 272. 1952.

(4) MacRae, H. F., and Baker, B. E. Application of Electrophoresis on Paper to the Estimation of Alpha-, Beta-, and Gamma-Casein. J. Dairy Sci., 41: 233. 1958.

(5) Marier, J. R., Tessier, H., and Rose, D. Sialic Acid as an Index of the κ-Casein Content of Bovine Milk. J. Dairy Sci., 46: 373. 1963.

(6) McKenzie, H. A., and Wake, R. G. I. Some Observations on the Heterogeneity of Casein Fractions. Australian J. Chem., 12: 712. 1959.

(7) McMeekin, T. L. The Proteins. Vol. IIa, p. 389. ed. Neurath, H. and Bailey, K. Academic Press, New York. 1953.

(8) McMeekin, T. L., and Polis, B. D. Advances in Protein Chemistry. Vol. V, p. 205. 1949.

(9) Morr, C. V. Investigating β-Lactoglobulin and κ-Casein Solutions and Their Interaction by Means of Fluorescence Polarization. Ph.D. thesis, Ohio State University, 1959. Dissert. Abstr., 20: 3943. 1960.

(10) Rose, D., Tessier, H., and Marier, J. R. Fractionation of κ-Casein on a DEAE Cellulose Column. (In press.)

(11) Sode-Mogenson, M. T., and Lahav, E. Observations on the Examination of Casein Preparations by Paper Electrophoresis. Lab. Practice, 9: 21. 1960.

(12) Swaisgood, H. E., and Brunner, J. R. Characterization of κ-Casein Obtained by Fractionation with Trichloracetic Acid in a Concentrated Urea Solution. J. Dairy Sci., 45: 1. 1962.

(13) Waugh, F. D. The Interaction of α_s-, β-, and κ-Caseins in Micelle Formations. Faraday Soc., Disc. No. 25. 1958.

(14) Waugh, F. D. Casein Interaction and Micelle Formation. J. Phys. Chem., 65: 1793. 1961.

(15) Zittle, C. A. Procedure for Isolation of κ-Casein by Use of Sulfuric Acid. J. Dairy Sci., 45: 650. 1962.

(16) Zittle, C. A., Cerbulis, J., Pepper, L., and Della Monica, E. S. Preparation of Calcium-Sensitive α-Casein. J. Dairy Sci., 42: 1897. 1959.

(17) Zittle, C. A., and Pepper, L. Influence of Hydrogen and Calcium Ion Concentration, Temperature, and Other Factors on the Rate of Aggregation of Casein. J. Dairy Sci., 41: 1671. 1958.

VOLATILE COMPOUNDS IN LADINO CLOVER AND OFF-FLAVORED MILK[1, 2]

A. E. WOODS[3] AND L. W. AURAND
Department of Food Science, North Carolina State College, Raleigh

SUMMARY

A low-temperature, high-vacuum distillation method, with liquid nitrogen trapping, was used to separate the volatiles from milk. The carbonyls were then precipitated as the 2,4-dinitrophenylhydrazones, washed, and dried. The carbonyls were regenerated by flash exchange, fractionated by gas liquid chromatography, and identified by retention times. Ethanal, acetone, and butanone were present in normal milk. In addition, 2-methylpropanal and 3-methylbutanal were present in off-flavored milk. Quantitative determinations of the individual compounds were performed. Steam distillates from Ladino clover were analyzed to determine the identity of the volatile components in the clover which may impart an off-flavor to milk. The volatiles were identified as carbonyl compounds and were fractionated in the same manner as above. Ethanal, acetone, butanone, pentanal, 2-methylpropanal, and 3-methylbutanal were present in all samples. Propanal was present in winter and summer samples of Ladino clover. Butanal was present in only spring clover.

Ladino clover fed with either fescue or orchard grass constitutes an important part of the winter pasture program of many southern dairymen. The need for these green forages is increased in early spring. Ironically, the dairymen have been limited in the extent to which these feeds can be used, because an off-flavor is imparted to the milk during this period of time. Little information is available regarding the chemical nature of the volatile components. Consequently, studies were made of the composition of the volatile components in Ladino clover and the volatile components in the off-flavored and normal flavored milks.

EXPERIMENTAL PROCEDURES

Ladino clover. Samples were collected throughout the growing season from a pure stand of Ladino clover located on the college farm. The samples were extracted immediately or were stored at −20 C until time of analysis.

Off-flavor milk. A switchback type of feeding experiment was used to produce the off-flavored milk. Four Holstein cows were selected for the experiment. Two of the
were placed on diets of hay and grain.
milk from these animals served as the co
The two remaining cows were pastured
Ladino clover–orchard grass pasture. A
end of a 2-wk feeding trial, the diets o
two pairs were reversed. The cows were n
twice each day by hand. The samples
taken to the laboratory (15-20 min), cool
7 C, and processed within a 24-hr period.
morning milks and the evening milks
pooled.

Steam volatile components from clover.
Ladino clover (500 g) was blended wit
tilled water (1,000 ml) in a 1-gal ca
Waring Blendor. The slurry was poured
a flask and the flask attached to a simple
distillation system consisting of a steam
erator, distillation flask, condenser, ic
cooled trap, and a 2,4-dinitrophenyl (
hydrazine trap. The gas trap containing
hydrazine reagent (200 mg in 20 ml co
trated hydrochloric acid) was used to e
that all carbonyl compounds were tra
The distillate was treated with the DN
drazine reagent. The turbid suspension
allowed to stand at room temperature f
hr. The DNP hydrazones were collecte
centrifugation, washed with 2 N hydroc
acid and water, and dried to constant wei
reduced pressure over phosphorous pent

Volatile components from milk. Isolati
the volatiles from milk was accomplished

Received for publication March 7, 1963.

---

[1] Authorized for publication as Paper No. 1582 in the Journal Series of the North Carolina Agricultural Experiment Station.

[2] This investigation was supported in part by a grant from the American Dairy Association.

[3] Present address: Chemistry Department, Middle Tennessee State College, Murfreesboro.

a high-vacuum, low-temperature distillation apparatus (Figure 1). Ten liters of cold (7 C) sample were placed in a 12-liter distilling flask and the flask attached to the system. The pressure was slowly lowered to 750 μ of mercury. After a pressure of 750 μ was reached, heat was applied to the distilling flask by means of a heating mantle. The temperature was not permitted to exceed 30 C. A distillation period of 2 hr was used for the removal of the carbonyls from the samples.

Studies had indicated the flavor volatiles were in the first of the two liquid nitrogen traps (Figure 1). Consequently, this trap was removed from the system, 30 ml of DNP hydrazine reagent added, and the trap then slowly withdrawn from the coolant so that the volatiles could react with the DNP hydrazine reagent. The reaction mixture was allowed to stand overnight. The precipitate was centrifuged, washed, and dried as previously described.

Fractionation of carbonyl derivatives. A Perkin-Elmer Fractometer Model 154 B, equipped with a thermal conductivity detector, was used for the analyses of the carbonyls regenerated from the DNP hydrazones. The columns were of stainless steel (¼ in. by 6 ft) with 20% w/w diisodecylphthalate on 60-80 mesh Celite. The operating conditions employed in the partition of the carbonyls were a temperature of 82 C, a column pressure of 10 psi, and a helium gas flow rate of 32 ml/min. The carbonyl compounds were regenerated from their DNP hydrazones and introduced into the column by the method described by Ralls (2, 3).

Identification of carbonyl derivatives. Tentative identification of the fractionated carbonyls was obtained by comparing their retention times with those of authentic compounds. Conclusive identifications were obtained by comparing the infrared spectra of the DNP hydrazones of the unknowns and authentic compounds. For recovery of the fractional carbonyls the resolved carbonyls were trapped at the exit port of the fractometer in DNP hydrazine solution. The DNP hydrazones were washed, dried, then pressed into potassium bromide pellets for infrared analysis.

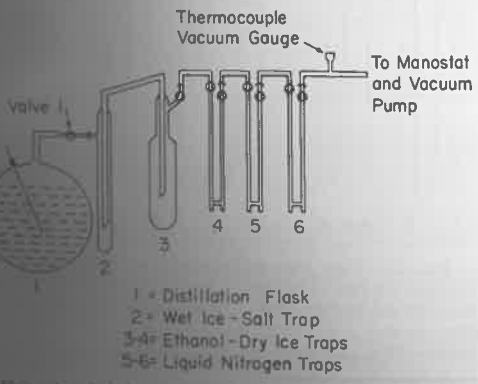

Thermocouple Vacuum Gauge
To Manostat and Vacuum Pump
Valve 1
1
2
3
4
5
6
1 = Distillation Flask
2 = Wet Ice - Salt Trap
3-4 = Ethanol - Dry Ice Traps
5-6 = Liquid Nitrogen Traps

FIG. 1. High-vacuum low-temperature distillation apparatus.

Estimation of carbonyl derivatives. For quantitative evaluation, the procedure for obtaining the DNP hydrazones and fractionation of the carbonyls was the same as the procedure outlined above. The DNP hydrazones were dried and weighed before chromatographic analysis. To determine the quantities of carbonyl present in the sample, the following formula was used:

$$\text{Concentration of carbonyl in } \mu\text{g/liter} = \frac{R \times T_h \times MW_c \times 1{,}000}{MW_h}$$

R = Ratio of the area under the peak for free carbonyl to the total areas of the carbonyls

T_h = Total weight of the DNP hydrazones in milligram per liter

MW_c = Molecular weight of the carbonyl

MW_h = Molecular weight of the hydrazone

RESULTS AND DISCUSSION

The milk from cows pastured on Ladino clover–orchard grass possessed an off-flavor which gradually decreased in intensity from early April to June. Preliminary studies indicated that the flavor of milk was always more bland following distillation than before the treatment, indicating the flavor was volatile in nature. Subsequent studies showed the volatile flavor components were carbonyl compounds.

A typical chromatogram of the volatile carbonyls in a high-quality milk obtained from cows on the hay-grain control ration showed ethanal, acetone, and butanone to be present (Figure 2A). Bassette and Whitnah (1) observed that high-quality winter milk, produced on a hay-grain ration, contained acetone and butanone. Wong and Patton (5) reported the presence of methanal, ethanal, acetone, butanone, pentanone-2, and hexanone-2 in fresh whole milk. Wishner and Keeney (4) isolated methanal, ethanal, acetone, and butanone from pasteurized whole milk. Thus, it appears that even though some variation occurs in the volatile carbonyls present in high-quality milk the chromatograms might be used objectively to evaluate the flavor quality of a milk.

The milk from cows pastured on Ladino clover–orchard grass was subjected to the same analytical procedure as the milk from the con-

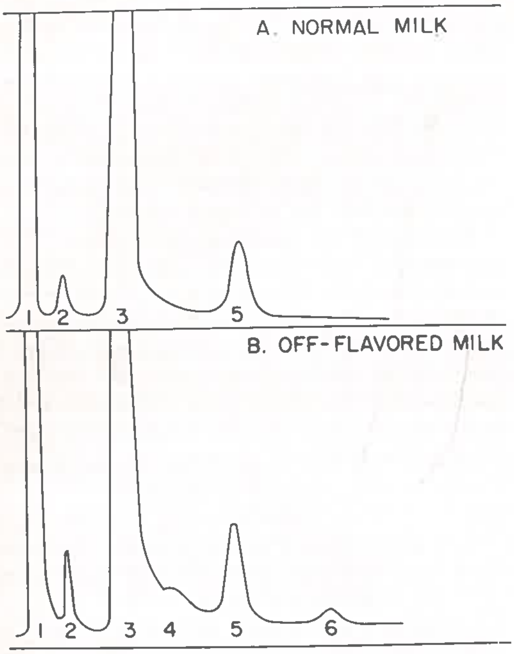


FIG. 2. Gas chromatograms of volatiles from normal and off-flavored milk. Column temperature 82 C, Helium flow rate 32 ml per minute, Column-diisodecylphthalate. The peaks represent air (1), ethanal (2), acetone (3), 2-methylpropanal (4), butanone (5), 3-methylbutanal (6). Ordinate represents recorder response, abscissa represents time.

trol group. Gas chromatograms of the milk produced by cows on summer (June) Ladino clover–orchard grass were the same as shown in Figure 2A. In contrast, the off-flavored milk produced by cows on spring Ladino clover–orchard grass showed the presence of ethanal, acetone, butanone, 2-methylpropanal, and 3-methylpropanal (Figure 2B). Comparison of the two chromatograms show that ethanal, acetone, and butanone were present in both the normal and off-flavored milk; however, 2-methylpropanal and 3-methylbutanal were also
tected in the off-flavored milk.

Conceivably, the distinction between the
flavors could be a matter of relative conce
tions of the volatile carbonyls rather tha
presence or absence of particular carbo
Consequently, a quantitative evaluation
made of the volatile carbonyls present in
the normal and off-flavored milks (Tabl
There was an over-all increase in concentr
of the carbonyls in off-flavored milk, bu
relative concentrations of ethanal, acetone
butanone were similar for both milks.
it appeared that 2-methylpropanal an

TABLE 1

Concentrations of carbonyls obtained from normal and off-flavored milk [a]

Compound	Normal milk		Off-flavored milk	
	(µg/liter)	*(%)*	*(µg/liter)*	*(%)*
Ethanal	2.9	2.6	4.3	2.9
Acetone	95.7	88.3	122.1	83.1
2-Methylpropanal	0.0	0.0	4.3	2.9
Butanone	9.8	9.1	15.2	10.2
3-Methylbutanal	0.0	0.0	1.3	0.9

[a] Six different samples of each kind of milk.

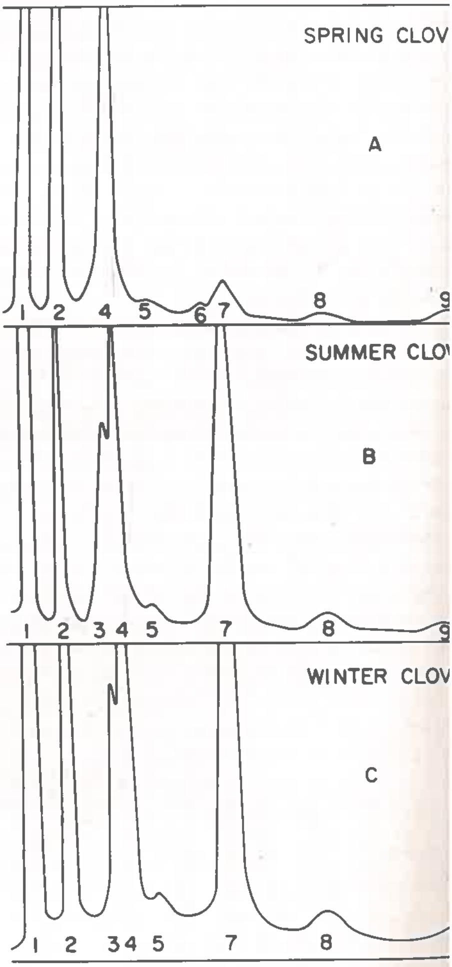
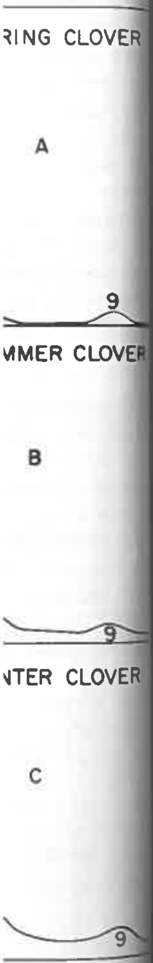


FIG. 3. Gas chromatograms of volatiles
Ladino clover. Column temperature 82 C,
flow 32 ml per minute, Column-diisodecylpht
The peaks represent air (1), ethanal (2)
panal (3), acetone (4), 2-methylpropana
butanal (6), -butanone (7), 3-methylbutana
and pentanal (9). Ordinate represents re
response and abscissa represents time.

were also d

etween the t
ative concentr
rather than t
ular carbony
evaluation w
present in b
ilks (Table 1
in concentrati
d milk, but t
al, acetone, a
h milks. Th
opanal and



of volatiles fr
ure 82 C, Heli
isodecylphthal
thanal (2), p
hylpropanal (
ethylbutanal (
presents recor
time.

---

methylbutanal were the volatile carbonyls responsible for the off-flavor.

It was of interest to study the change, if any, in volatile carbonyls of the Ladino clover during the spring, summer, and winter seasons. Chromatograms of the various samples showed that ethanal, acetone, pentanal, 2-methylpropanal, butanone, and 3-methylbutanal were common to all samples (Figure 3). Propanal was present in the winter and summer clovers, while butanal was present only in the spring clover. There was no difference in the relative concentration of the other components with season (Table 2). Ethanal, acetone, and butanone comprised the major portion of the volatile components in all samples. The volatile carbonyls, 2-methylpropanal and 3-methylbutanal, were present in all Ladino clover samples. Thus, the composition of the feed does not appear to be related directly to the flavor components in the milk. Presumably, many factors are involved in the flavor of milk, not the least of which would be the rumen.

TABLE 2

Distribution of volatiles from Ladino clover of different seasons [a]

Compound	Spring	Summer	Winter
	(relative per cent)		
Ethanal	41.3	20.2	20.2
Propanal	0.0	16.2	11.4
Acetone	45.9	16.1	24.8
2-Methylpropanal	1.1	1.0	0.7
Butanal	1.8	0.0	0.0
Butanone	6.8	43.2	37.9
3-Methylbutanal	0.9	2.1	3.8
Pentanal	2.2	0.8	1.3

[a] Six different samples of each kind of Ladino clover.

REFERENCES

(1) Bassette, R., and Whitnah, C. H. Mass Spectrometry and Gas Chromatography Applied to the Study of Volatiles in Milk. J. Dairy Sci., 44: 1164. 1961.

(2) Ralls, J. W. Rapid Method for Semiquantitative Determination of Volatile Aldehydes, Ketones, and Acids. Anal. Chem., 32: 332. 1960.

(3) Ralls, J. W. Flash Exchange Gas Chromatography for the Analysis of Potential Flavor Components of Peas. Agr. Food Chem., 8: 141. 1960.

(4) Wishner, L. A., and Keeney, M. Some Carbonyl Formed by the Action of Sunlight on Milk. J. Dairy Sci., 44: 1164. 1961.

(5) Wong, N. P., and Patton, S. Identification of Some Volatile Compounds Related to the Flavor of Milk and Cream. J. Dairy Sci., 45: 724. 1962.

DETERMINATION OF HEAT-INDUCED LACTOSE-PROTEIN COMBINATION IN MILK WITH LACTOSE-1-C^{14}

E. K. NIELSEN, R. A. MONROE, AND R. V. HUSSONG
Fundamental Laboratory, Research and Development Division
National Dairy Products Corporation, Glenview, Illinois

SUMMARY

A tracer study was made to determine how much of the lactose in cow's milk combined with the protein fraction on heating. This was done by adding an aqueous solution of lactose-1-C^{14} to fresh skimmilk, allowing it to equilibrate before heating, isolating protein, by acid precipitation or exhaustive dialysis, and counting the C^{14} in the isolate. Protein isolated by either method contained C^{14} tracer, indicating a protein-lactose interaction to form a stable complex. The protein fraction of raw skimmilk was found to contain 0.03% of the added tracer. Heating the dosed skimmilk at 145 and 208 F for 30 min resulted in the presence of 0.06 and 0.7% of added tracer in the protein fraction. These values, then, represented the percentages of lactose, either in the form of intact lactose or radioactive fragments thereof, combined with the protein as a result of the heat treatments accorded.

Studies of Kass and Palmer (4), and Gould (1), have shown that considerable destruction of lactose occurs in milk when it is heated for prolonged periods at elevated temperatures. Grimbleby (2) demonstrated that there was an inverse relationship between the titratable acidity and formol titer of raw separated milk heated at temperatures of 60, 70, and 80 C. These results suggested that the combination of lactose and protein, with the elimination of basic amino groups attached to protein, is one of the main reactions responsible for heat-induced acidity. Patton and Flipse (5) established that when lactose-1-C^{14} was added to raw milk which was then heated, a portion of the added radioactivity became bound by the protein. The amount of radioactivity bound was directly related to the extent of heat treatment. The objectives of the present study were to extend this observation to heat treatment at lower temperature and confirm their finding by using the more efficient scintillation counting technique, since made available, instead of planchet counting.

EXPERIMENTAL PROCEDURE

A pint of fresh, uncooled milk from the morning milking of Holstein cows was preserved with about 5 ml of toluene and promptly brought to the laboratory. Most of the fat was separated from the milk by centrifuging in 250-ml bottles with a No. 2 International Centrifuge at 1,700 rpm for 5 min. Skimmilk was withdrawn from below the fat layer with pipette and transferred to 40-ml centrifu tubes. Recentrifugation removed traces of f Forty-nine milliliters of this skimmilk, tran ferred to a 125-ml Erlenmeyer flask, was dos with 500 μl of lactose-1-C^{14} solution containin an activity of 2.196×10^6 disintegrations p minute (DPM) per milliliter, and follow with one 500-μl water rinse of the pipette. T mixture was agitated and allowed to stand 1 hr at room temperature. Fifteen milliliters this dosed skimmilk was transferred into ea of two 20-ml vials, which were then closed wi screw caps. One vial was held in a 145 F wat bath for 30 min. The other was held in a boi ing water bath at 208 F for half an hou After these were cooled to room temperatur two 5.0-ml aliquots of each of the raw a heat-treated milks were pipetted into separa dialysis bags. Two drops of toluene were adde to the contents of each bag. These bags h been formed by tying a knot in one end of 8-in. length of cellulose dialyzing tubing (in. in diameter) with an average pore size 48 Angstrom units and a thickness of 0.0008 The six dialysis bags containing the 5.0- aliquots were attached, by means of cellopha tape, to a rod suspended vertically from a ri stand. The assembly was lowered, so that dialysis bags extended 3 to 3.5 in. into 500 of phosphate buffer contained in a beak The buffer was M/50 in both $Na_2HPO_4 \cdot 7H$ and $NaH_2PO_4 \cdot H_2O$. The beaker contained 1-in. polyethylene-coated magnetic stirring and rested on a variable speed magnetic stir

Received for publication March 11, 1963.

base. The speed of the stirrer was adjusted to produce movement of the buffer solution around the dialysis bags. The whole unit was held in a 45 F cooler during the period of dialysis. Fresh buffer was placed in the beaker at successive intervals of 18, 8, 16, and 8 hr. The final dialysis period was 16 hr. Contents of the dialysis bags were transferred to special 20-ml counting vials (Packard Instrument Co., LaGrange, Illinois). These were placed in a hot water bath and most of the water evaporated from the samples. Each residue was treated with 1.2 ml of Hyamine 10-X solution (Packard Instrument Co.) and the vial capped. Intermittent warming in a hot water bath below 140 F and agitation on a Vortex Mixer (Scientific Industries, Inc., Springfield, Mass.) brought the residues into solution. Ten milliliters of scintillation solvent was added to each vial. The scintillation solvent contained 150 g of naphthalene, 30 g of 2,5-diphenyloxazole (PPO), 1.5 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP), 500 ml cellosolve, and 2,500 ml dioxane, each of reagent grade. The mixture was acidified with 10% v/v H_2SO_4 in ethanol, to obviate difficulties from phosphorescence which sometimes occur in protein-Hyamine solution (3). Capped vials were placed in a Tri-Carb Liquid Scintillation Spectrometer (Series 314A, Packard Instrument Co.), cooled, and counted for several 30-min periods. To obtain the background count, a vial containing 1.2 ml Hyamine 10-X, 10 ml scintillation solvent, and acidified with ethanolic sulfuric acid was placed in the Tri-Carb and counted along with and under the same conditions as the experimental samples. To determine the fraction of lactose tracer which was nondialyzable, or in combination with the protein fraction, it was necessary to know exactly the activity of the lactose-1-C^{14} added to skimmilk, as well as that remaining in the sample. The addition of an internal standard, toluene-C^{14}, served to determine counting efficiency and, in each case, compensated for internal differences in the samples. The efficiency values allowed direct comparison of sample counts by conversion of the net count per minute (CPM) to disintegrations per minute (DPM). Then the activity remaining in the dialyzed sample could be expressed directly as per cent of the activity of the lactose-1-C^{14} initially added.

RESULTS AND DISCUSSION

Since an aqueous solution of the lactose-1-C^{14} was added to the skimmilk, and the dosed milk mixed and allowed to equilibrate 1.5 hr, it was assumed that the behavior of the added lactose-1-C^{14} reflected the behavior of all the lactose in the dosed skimmilk. Preliminary experiments indicated that heating of skimmilk containing added lactose-1-C^{14} resulted in an increase of radioactivity in protein which was precipitated at pH 4.6, soaked, and washed several times with water. To lessen the possibility of occluding lactose-1-C^{14} in the precipitate, thus leading to an erroneous observation, it was decided to remove the lactose-1-C^{14} by exhaustive dialysis. The data obtained in these latter experiments confirmed the earlier observations and are presented in Table 1.

Pasteurization, 145 F for 30 min, of the skimmilk doubled the nondialyzable radioactivity found in the similar fraction of raw skimmilk; namely, from 0.03 to 0.06% of the added radioactivity. Heating the skimmilk to 208 F for 30 min gave a tenfold increase in the nondialyzable radioactivity over that found in the identical fraction of pasteurized skimmilk.

Grimbleby (2), in his studies on heat-induced acidity in milk, determined polarimetrically the effect of heat on the lactose content of raw separated milk. His results showed a loss of 1.5% of the lactose when separated milk was heated to 100 C for 0.5 hr. In our experiments, similar heating, 208 F (98 C) for 30 min, Table 1, showed that 0.68% of the added radioactivity was nondialyzable. This accounts for about half of the lactose destroyed, as measured by Grimbleby (2). That is to say, possibly only a fraction of the lactose destroyed by heat was accounted for by radioactivity in the nondialyzable fraction. On the other hand, it is possible that nonlabeled fragments of lactose could have combined with protein undetected by our methods.

While Patton and Flipse (5) found that slightly more than 1% of the radioactivity from added lactose-1-C^{14} was bound by protein when whole milk was heated at 97 C for 30 min, the current study shows that 0.7% was bound when skimmilk was heated at 98 C for 30 min. Possible explanations for this difference may be: (a) variations in the medium used for experimental purposes, (b) variations in heat treatment, since Patton and Flipse (5) do not indicate whether time to reach the indicated temperature was considered part of the total heat treatment, or (c) use of a more precise measuring device in scintillation counting as compared to planchet counting.

The nondialyzable nature of the radioactivity remaining in the heated skim, as compared to the readily dialyzable nature of the lactose-1-C^{14} added, indicated that a relatively large molecule had been formed with (or from) a

TABLE 1

Heat-induced lactose-protein combination in skimmilk as studied by use of added[a] radioactive tracer, lactose-1-C^{14}

Treatment	Raw skimmilk		Heated skimmilk			
			(145 F-30 min)		*(208 F-30 min)*	
Sample no.	1	2	1	2	1	2
DPM[b]	321	396	654	596	7,570	7,358
Per cent of activity added	0.029	0.036	0.060	0.054	0.69	0.67

[a] The activity added per 5.0-ml sample used was determined on the dosing lactose solution and found to be 1.098 × 10^6 DPM.

[b] DPM was determined by correcting the observed net count rate for the counting efficiency, as indicated by the addition of certified C^{14} standard.

relatively small molecule, lactose. Neither the nature of the complex, nor the mechanism by which it is formed, is revealed in this study. Whatsoever the combination, or the manner in which it is formed, it is evident from this tracer study that the compound formed is derived from and involves the added lactose-1-C^{14}. Furthermore, since the preliminary experiments with acid precipitation of protein from heated skimmilk which had been treated with lactose-1-C^{14} gave the same results, it is concluded that the complexing took place with the protein. The identical results obtained under these different conditions of protein isolation point to the relative stability of the complex under conditions of dialysis.

ACKNOWLEDGMENTS

The authors thank Mr. Arthur Kontrovitz for his technical assistance in the analysis of t samples and Dr. E. H. Marth for reviewing t manuscript.

REFERENCES

(1) Gould, I. A. Lactic Acid in Dairy Produc III. The Effect of Heat on Total Acid a Lactic Acid Production and on Lactose struction. J. Dairy Sci., 28: 367. 1945.

(2) Grimbleby, F. H. Heat-Induced Acidity Milk. J. Dairy Research, 21: 207. 195

(3) Herberg, R. J. Phosphorescence in Liqu Scintillation Counting of Proteins. Scien 128: 199. 1958.

(4) Kass, J. P., and Palmer, L. S. Browni of Autoclaved Milk. Ind. Eng. Chem., 3 1360. 1940.

(5) Patton, S., and Flipse, R. J. Studies Heated Milk. V. The Reaction of Lact with Milk Protein as Shown by Lactose-C^{14}. J. Dairy Sci., 36: 766. 1953.

GERMINATION RESPONSE OF *BACILLUS LICHENIFORMIS* SPORES TO AMINO ACIDS[1]

J. H. MARTIN AND W. J. HARPER

Department of Dairy Technology, The Ohio State University, Columbus

SUMMARY

Twenty-four amino acids were studied as to their effect on germination of *Bacillus licheniformis* spores, and only L-alanine, L-cysteine, and L-valine markedly stimulated germination. Considerable variation in the rate and extent of germination in response to L-cysteine and L-valine was observed with several isolates of *B. licheniformis* obtained from raw milk, but all isolates responded similarly to L-alanine. The rate at which germination occurred in the presence of these amino acids with one particular isolate of *B. licheniformis* was extremely rapid, with significant germination evident after 4 min of incubation with L-alanine, after 6 min with L-cysteine, and after 8 min with L-valine. Very low levels of L-alanine were sufficient to stimulate considerable germination of *B. licheniformis* spores, with 0.0005 and 0.001% L-alanine inducing 40 and 68% germination, respectively, after 15 min of incubation at 35 C. D-alanine was only slightly stimulatory to spore germination, and when present with equal concentrations of L-alanine, the stimulatory effect of L-alanine was inhibited.

Spore-forming microorganisms in the modern milk supply are significant because of their prevalence, their relatively high heat resistance, and because of current trends in the dairy industry towards higher pasteurization temperatures and extended storage times for both raw and pasteurized dairy products.

Recently, spore-forming bacilli were found in 285 of 287 raw milks in Ohio (11). *Bacillus licheniformis* was the most frequently occurring spore-forming organism, being present in 43% of the milk samples. This particular organism has received only limited attention (17, 19), with no information available concerning intraspecies variations. Studies on other members of the genus *Bacillus* have revealed that amino acids most frequently satisfy germination requirements of bacterial spores (10, 16), but marked species variations in germination response to specific amino acids have been reported (16-17), and some intraspecies differences also have been observed (17).

This study was undertaken to determine the amino acid requirements for germination of *B. licheniformis* spores, and to ascertain if any intraspecies variation might exist in isolates obtained from raw milk.

METHODS

Preparation and standardization of spore suspensions. Isolates of *B. licheniformis* were obtained from different samples of raw milk. Spores were produced by inoculating actively growing vegetative cells onto Nutrient Agar (Difco) slants containing 1.0% starch and 0.01% manganese sulfate (2, 6). Greater than 90% sporulation of all isolates occurred after four days of incubation at 35 C.

The spores were washed from the agar slants with sterile M/100 phosphate buffer (pH 7.2), and concentrated by centrifugation at 3,000 rpm in an International Model S-1 centrifuge. The spores were resuspended in phosphate buffer and the washing and centrifugal steps repeated four times. The washed spores were resuspended, heated at 80 C for 10 min to eliminate viable vegetative cells, and centrifuged a fifth time before the suspension was standardized. This procedure provided clean spore suspensions, as evidenced by the absence of vegetative cells and cell debris by (a) phase contrast microscopy and (b) light microscopy of crystal violet–stained smears (4).

Spore suspensions in M/100 phosphate buffer were standardized turbidimetrically to 58% light transmission, using a Bausch and Lomb Spectronic 20 Colorimeter at a wave length of 725 mμ. This provided a suspension of $10^8 \pm 10\%$ spores/ml. Spore suspensions were used within 24 hr after preparation.

Received for publication April 12, 1963.

[1] Article Number 7-63. Department of Dairy Technology, Ohio State University. Supported by a grant from the U. S. Public Health Service (National Institutes of Health).

Determination of germination response to amino acids. Two methods were utilized for determining spore germination: (a) a qualitative procedure involving the increase in per cent light transmission of spore suspensions upon germination (7), and (b) a quantitative plate count procedure based on the loss of heat resistance of germinated spores (1, 18). The latter method employed the dilution heat-shock technique outlined by Fernelius (5), in which 1 ml of the spore suspension was added to 99 ml of sterile distilled water which had been tempered to 80 C in a water bath before the addition of spores. Heating at 80 C was continued for 10 min, and the ungerminated spores surviving this heat treatment were enumerated on Plate Count Agar (Difco) containing 0.1% soluble starch (15) after incubation at 35 C for 18 hr.

The amino acids were added to the spore suspensions as 10% solutions in 2 N HCl, the pH was adjusted to 7.2 with 0.1 N NaOH, and phosphate buffer was added to give a final concentration of 0.1% amino acid. This mixture was incubated at 35 C and aliquots were removed at 0-, 15-, 30-, 45-, 60-, and 120-min intervals to determine germination. For comparison, a control sample was used to which no amino acid was added.

RESULTS

Germination response to various amino acids. A study was made of the effects of 24 amino acids on the germination of spores of *B. licheniformis* isolates, utilizing optical density measurements. Amino acids which did not stimulate germination were: DL-alanine, L-arginine, D-asparagine, L-asparagine, D-aspartic acid, L-aspartic acid, D-cysteine, L-cystine, L-glutamic acid, D-glutamic acid, glycine, L-histidine, D-methionine, L-methionine, L-proline, D-phenylalanine, L-phenylalanine, D-tryptophane, L-tryptophane, and D-valine.

The amino acids L-alanine, L-cysteine, and L-valine markedly stimulated germination, and D-alanine was slightly stimulatory. Results obtained with one isolate of *B. licheniformis* utilizing these amino acids are presented in Figure 1. Germination occurred very rapidly in response to L-alanine, L-cysteine, and L-valine, with 92, 85, and 70% germination, respectively, occurring after 15 min of incubation. The response of the spores to D-alanine was low, with only 5% germination occurring during 15 min. The total level of germination essentially was complete after the first 15-min period, with less than 10% increase during the remainder of the incubation period with L-alanine, L-cysteine, and L-valine. The total level of germinati was reached after approximately 1 hr of in bation in the presence of D-alanine.

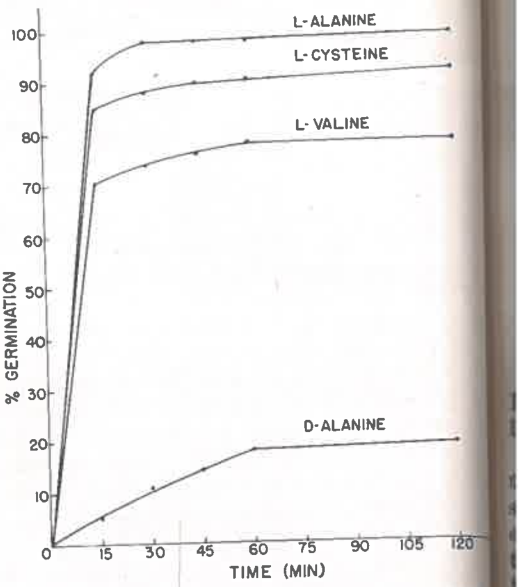


FIG. 1. Germination of spores of *B. lich formis A* in response to L-alanine, D-alanine, cysteine, and L-valine.

To determine whether different isolates fr raw milk would respond similarly to sti latory amino acids, the germination respo of seven isolates of *B. licheniformis* to 0.1 L-alanine, L-cysteine, and L-valine was det mined. Results obtained are presented in Ta 1. These data show the germination respo after 15 min as an index of the rate of g mination, and after 2 hr of incubation as index of total germination.

All isolates showed greater germination w L-alanine than with L-cysteine or L-valine. C siderable variation occurred in germination tween isolates with L-cysteine and L-valine. general, with the exception of isolate C, g mination response to L-alanine, both after min and 2 hr, was relatively constant. The i tial response of isolates to L-cysteine and valine was quite variable, ranging from 5.5 85.3% with L-cysteine, and from 18.8 to 70. with L-valine after 15 min of incubation.

It is evident from these data that the ger nation occurring during the first 15-min pe may serve as an index of total germination sponse with L-alanine, but it does not pro an index of total germination response to cysteine and L-valine for all isolates stud Isolates B, C, and D germinated consider in response to L-cysteine following the ini

TABLE 1

Germination response of different isolates of *B. licheniformis* to amino acids[a]

Isolate	Per cent germination after 15 min of incubation			Per cent germination after 2 hr of incubation		
	L-alanine	L-cysteine	L-valine	L-alanine	L-cysteine	L-valine
A	91.9	85.3	70.5	98.8	91.5	77.6
B	86.2	33.2	18.8	93.0	62.5	43.6
C	70.0	5.5	21.5	97.4	21.6	43.8
D	90.8	28.1	23.7	95.8	51.5	61.3
E	91.8	66.7	27.1	95.7	79.7	57.0
F	93.2	47.0	59.6	97.4	61.5	77.0
G	90.7	46.2	23.9	97.9	64.2	45.3

[a] Average of three to five trials with each isolate.

15-min period, and the same was true for isolates B, C, D, E, and G with L-valine.

Rate of germination response to L-alanine, L-cysteine, and L-valine. To determine the response of spores to amino acids during the early stages of incubation, a temperature-controlled (35 C) roto-cell was attached to the Spectronic 20 Colorimeter, and measurements of the changes in per cent light transmission were made at 1-min intervals. Observations indicated that a change of at least 1% light transmission had to occur before there was a significant loss of heat resistance of the spore suspensions. Therefore, significant germination was considered to have begun when more than 1% increase in light transmission had occurred. The results (Table 2) reveal that germination of *B. licheniformis A* was initiated after 4, 6, and 8 min in the presence of L-alanine, L-cysteine, and L-valine, respectively. Following initiation of germination, there was an increase in light transmission of approximately 1% per minute, irrespective of the amino acid.

Effect of D-alanine on the germination response of spores of B. licheniformis A to L-alanine. In many instances with other *Bacillus* species, D-alanine has been reported to be inhibitory to spore germination (16). Since D-alanine did induce slight germination of *B. licheniformis A,* the effect of D-alanine on the germination induced by L-alanine was determined. The germination response of *B. licheniformis A* to various concentrations of L-alanine, D-alanine, and equal mixtures of L- and D-alanine is presented in Table 3.

Very low levels of L-alanine resulted in significant germination of *B. licheniformis A* with 0.0005% producing approximately 40% germination within 15 min, and 79% after 2 hr of incubation. When 0.001% L-alanine was added,

TABLE 2

Rate of germination of spores of *B. licheniformis A* in response to amino acids as measured by change in light transmission[a]

Incubation time (*min*)	Control	Change in per cent transmission in response to		
		0.1% L-alanine	0.1% L-cysteine	0.1% L-valine
0	54.0	59.5	60	60
1	54.0	59.5	60	60
2	53.5	59.5	60	60
3	54.0	61.0	60	60
4	54.0	62.0	60.5	60
5	54.0	63.5	61.0	60
6	54.0	64.0	61.5	60.5
7	54.0	65.0	63.0	61.0
8	54.0	66.0	64.0	61.5
9	55.0	67.0	64.5	62.5
10	55.0	68.0	66.0	63.5
11	55.0	70.0	67.0	64.0
12	55.0	71.5	68.0	65.5
13	55.0	73.5	69.0	67.0
14	55.0	75.0	70.0	68.5
15	55.0	77.0	72.0	70.0

[a] Spore suspensions incubated at 35 C in the Spectronic 20 Colorimeter using a special roto-cell at a wave length of 725 mμ.

TABLE 3

Germination response of spores of *B. licheniformis A* to L-alanine, D-alanine, and to equal mixtures of L- and D-alanine[a]

Incubation time *(min)*	Per cent germination in response to 0.0005% L-alanine	0.001% L-alanine	0.1% L-alanine	0.1% D-alanine	0.05% L-alanine +0.05% D-alanine	0.1% L-alanine +0.1% D-alanine
0	0	0	0	0	0	0
15	39.8	67.6	91.9	5.2	9.4	7.7
30	53.4	83.3	98.1	10.9	14.2	3.8
45	72.9	83.7	98.1	13.5	12.9	6.9
60	78.8	87.0	98.3	17.7	16.1	10.5
120	79.3	87.2	98.8	19.3	24.9	14.7

[a] Average of three trials with each concentration.

germination response was almost doubled during 15 min of incubation (68%), and in the presence of 0.1% L-alanine approximately 92% of the spores had germinated during the same incubation time. There was a marked difference in the germination response to L- and D-alanine, with approximately 99% germination after 2 hr of incubation with 0.1% L-alanine, and only 19% with 0.1% D-alanine. When equal mixtures of these amino acids were added, the D-alanine inhibited the germination response to L-alanine, with approximately 25% germination after 2 hr in response to 0.05% mixtures, and 15% with the 0.1% mixtures. These data indicate that D-alanine is not as readily utilized in spore germination as is L-alanine, and when D-alanine is present in a mixture with L-alanine, the stimulatory effect resulting from L-alanine is greatly reduced.

DISCUSSION

Results of this study reveal that the germination response of spores of *B. licheniformis* to amino acids is quite specific. Of the 24 amino acids studied, only L-alanine gave a uniformly high germination response with all of the isolates studied. The other amino acids which did cause germination, L-cysteine and L-valine, are similar to L-alanine in respect to their carbon chains. Further evidence of specificity is evident from the fact that D-cysteine, D-valine, and D-alanine either did not stimulate germination, or produced only a slight effect. The ability of D-alanine to inhibit germination response by L-alanine further supports the specificity of the reaction.

Results with other *Bacillus* species have shown that L-alanine is the most common amino acid capable of initiating germination in the species. L-alanine has been shown to promote germination of at least six different *Bacillus* species (10, 16, 17). In some instances, it has been necessary to have a riboside (inosine or
adenosine) or glucose in addition to L-alanin
to obtain germination. L-cysteine has been r
ported to promote germination of at least thr
Bacillus species (8, 10), and about the san
number have been shown to germinate in r
sponse to L-valine (10, 14).

The fact that L-alanine promotes germin
tion for many of the different *Bacillus* speci
suggests that it may act through a commo
mechanism. The slower and variable germin
tion response of spores of several isolates
B. licheniformis in the presence of L-cystei
and L-valine indicates that (a) germination
curs by a mechanism different from that
L-alanine, (b) the different isolates vary
their ability to transport L-cysteine or L-vali
across the spore coat, or (c) these amino aci
may be converted to L-alanine by an enzy
system active to a variable extent in the diff
ent isolates. Church and Halvorson (3) ha
suggested that spore enzymes may exist
either active, dormant, or inactive states in th
intact spore, and the poorer germination
sponse observed with L-cysteine and L-vali
may well be an indication of an activation ti
for essential enzymes.

The rapidity with which L-alanine initiat
germination in extremely low concentratio
should be considered in respect to the mec
anism by which spore germination occurs. T
of the most common theories are (a) that g
mination results from the triggering of
poised system, which suggests mainly an en
matic process (12), and (b) that a chel
form of dipicolinic acid–calcium-spore str
ture exists in the dormant spore, with break
of the chelate resulting in germination (1
If the spore structure involved in the chel
consisted of spore enzymes, then these
theories would seem to be similar. Results
this study support the proposal that a trig
ing reaction is involved, since germination

initiated almost immediately after the addition of L-alanine.

The findings of this investigation have possible practical application in the elimination of bacterial spores from milk without necessitating the high heat treatments currently required. The addition of suitable germination stimulants in low concentrations could provide initiation of germination within a relatively short period of time which would predispose the spores to destruction by moderate heat treatment. Conceivably, L-alanine could be an important adjunct in the additive system required to achieve this effect.

REFERENCES

(1) CAMPBELL, L. L., JR. Bacterial Spore Germination—Definitions and Methods of Study. Spores. p. 33. Ed., H. O. Halverson. Am. Inst. Biol. Sci., Washington, D. C. 1957.

(2) CHARNEY, J., FISHER, W. P., AND HEGARTY, C. P. Manganese as an Essential for Sporulation in the Genus *Bacillus*. J. Bacteriol., 62: 145. 1951.

(3) CHURCH, B. D., AND HALVORSON, H. Intermediate Metabolism of Aerobic Spores. I. The Activation of Glucose Oxidation in Spores of *Bacillus cereus* var. *terminalis*. J. Bacteriol., 73: 470. 1957.

(4) COLLIER, R. E. An Approach to Synchronous Growth for Spore-Production in *Clostridium roseum*. Spores. p. 10. Ed., H. O. Halvorson. Am. Inst. Biol. Sci., Washington, D. C. 1957.

(5) FERNELIUS, A. L. Comparison of 2 Heat-Shock Methods and a Phenol Treatment Method for Determining Germination Rates of Spores of *Bacillus anthracis*. J. Bacteriol., 79: 755. 1960.

(6) FOSTER, J. W., HARDWICK, W. A., AND GUIRARD, BEVERLY M. Antisporulation Factors in Complex Organic Media. I. Growth and Sporulation Studies on *Bacillus larvae*. J. Bacteriol., 59: 463. 1950.

(7) HACHISUKA, Y., ASANO, N., KATA, N., OKAJIMA, M., KITAORI, M., AND KUNO, T. Studies on Spore Germination. I. Effect of Nitrogen Source on Spore Germination. J. Bacteriol., 69: 399. 1955.

(8) KRASK, B. J. Spores II. p. 89. Burgess Publ. Co., Minneapolis, Minnesota. 1961.

(9) LEVINSON, H. S., AND HYATT, MILDRED T. The Stimulation of Germination and Respiration of *Bacillus megaterium* Spores by Manganese, L-alanine, and Heat. J. Bacteriol., 70: 368. 1955.

(10) LEVINSON, H. S., AND HYATT, MILDRED T. Nitrogenous Compounds in Germination and Postgerminative Development of *Bacillus megaterium* Spores. J. Bacteriol., 83: 1224. 1962.

(11) MARTIN, J. H., STAHLY, D. P., HARPER, W. J., AND GOULD, I. A. Spore-Forming Microorganisms in Selected Milk Supplies. Proc. XVIth Intern. Dairy Congr., 2: 295. 1962.

(12) O'CONNER, R. J., AND HALVORSON, H. O. The Role of Alanine Dehydrogenase in the Control of Germination of Aerobic Spores Initiated by L-alanine. Spores II. p. 73. Burgess Publ. Co., Minneapolis, Minnesota. 1961.

(13) REIMANN, H. Germination of Bacteria by Chelating Agents. Spores II. p. 24. Burgess Publ. Co., Minneapolis, Minnesota. 1961.

(14) SCHMIDT, C. F. Activators and Inhibitors of Germination. Spores. p. 56. Ed., H. O. Halvorson. Am. Inst. Biol. Sci., Washington, D. C. 1957.

(15) STAHLY, D. P. Heat Related Factors Affecting the Rate of Germination and Subsequent Growth of *Bacillus cereus* and *Bacillus licheniformis* Spores in Milk. M.S. thesis, The Ohio State University. 1961.

(16) STEDMAN, R. L. Biochemical Aspects of Bacterial Endospore Formation and Germination. Am. J. Pharm., 128: 84. 1956.

(17) THORLEY, C. M., AND WOLF, J. Some Germination Factors of Mesophilic Spore Formers. Spores II. p. 1. Burgess Publ. Co., Minneapolis, Minnesota. 1961.

(18) WILLIAMS, O. B. Symposium on the Biology of Bacterial Spores. Bacteriol. Revs., 16: 89. 1952.

(19) WOLF, J., AND MAHMOUD, S. A. Z. The Effects of L- and D-alanine on the Germination of Some *Bacillus* Spores. J. Appl. Bacteriol., 20: 373. 1957.

HOST-BACTERIOPHAGE RELATIONSHIPS AS INFLUENCED BY ANTIBIOTICS[1]

E. M. MIKOLAJCIK

Department of Dairy Technology, The Ohio State University, Columbus

SUMMARY

The effect of sublethal concentrations of selected antibiotics on the bacteriophage-host relationship of *Streptococcus lactis* C10 and bacteriophage c10 was studied by means of single-step growth curves.

The addition of penicillin, terramycin, and nisin to the adsorption mixture resulted in average burst sizes of 84, 16, and 30, respectively, compared to 42 bacteriophage particles per infected bacterium in the absence of antibiotics. The corresponding latent periods were 20, 24, 23, and 16 min.

The reproduction of new phage particles may be hastened or retarded by exposure of susceptible cells to sublethal concentrations of antibiotics.

Field observations have indicated a possible relationship between the exposure of lactic acid cultures to antibiotics and the subsequent susceptibility of the organisms to bacteriophage (phage) attack. Consequently, Richards et al. (8) investigated alterations in host-phage relationships due to sublethal concentrations of antibiotics and found that the rate of lysis of *Streptococcus lactis* C10 by phage c10 was altered by penicillin or terramycin, with the former hastening and the latter delaying cell destruction.

Additional studies were conducted to determine the underlying causes of such variations and this paper presents the results obtained.

EXPERIMENTAL PROCEDURE

The effect of penicillin, terramycin, and nisin on single-step growth curve characteristics of phage c10 was determined by using the procedure and calculations of Delbruck and Luria (6).

The *S. lactis* C10 organism and the phage c10 actively lysing this strain were those used by Richards et al. (8). In certain of the experiments, an induced penicillin-resistant variant of this strain served as the host. It was established that the phage sensitivity pattern of the resistant variant was not altered by the antibiotic treatment.

Sobol's broth (10) was used for carrying lactic cultures and as a stock medium for phage. The composition of this broth is: yeast extra 4.0 g; K_2HPO_4, 4.0 g; neopeptone (Difco 2.0 g; peptonized milk (Difco), 2.0 g; mil protein hydrolysate (BBL), 4.0 g; lactose, 4 g; distilled water, 1,000 ml; pH 6.8. Steril $CaCl_2$, to give a final concentration of 0.33 was added aseptically to this broth just befor inoculation.

An 16-18 hr culture of the bacterial cells w transferred and then incubated for 2 hr befor use. The 2-hr incubation period was used b cause (a) lysis was depressed with cells inc bated over 8 hr, and (b) *S. lactis* C10 cel were in the accelerated growth phase approx mately 2 hr after transfer into fresh broth. A the conclusion of the 2-hr incubation perio the viable cell count was determined by Stan ard Methods (3). The viable cell counts we used to calculate multiplicity of infection.

Plaque counts were determined by the doubl agar layer technique (1), using trypticase s agar (BBL) containing 0.02% L cystine (5 A 3-4 hr growth of the C10 organisms in brot was used for the seed layer.

Where antibiotics were used, penicillin, te ramycin, and nisin were added, just prior t adsorption, at the rates of 0.05 IU/ml, 0 μg/ml, and 10 μg/ml, respectively. These con centrations were found to have no significa influence on the growth rate of the host cells

The adsorption tube mixture consisted of th 2-hr culture of the host cells and an aliquot o phage of known titer. Multiplicity of infecti was 0.1. The adsorption mixture was incubat at 30 C for 5 min. An aliquot was then wit drawn and diluted with fresh broth to prev further adsorption. The diluted cell-phage m ture was incubated at 30 C and plaque cou

Received for publication October 26, 1962.

---

[1] Article 8-62, Department of Dairy Technology. Supported by a grant from the U. S. Public Health Service (National Institutes of Health).

were made on aliquots withdrawn at 2-min intervals until the phage titer showed no further increase.

The remaining portion in the adsorption tube was centrifuged immediately for 5 min at 5,000 rpm. The per cent adsorption was determined by the difference between the initial titer and the unadsorbed phage present in the supernatant. For comparison, single-step growth curves were prepared for host-phage systems which contained no antibiotic.

RESULTS

Results obtained for phage adsorption, burst size, latent period, and rise period are presented in Table 1.

The data reveal rather wide variations between trials, especially for adsorption and burst size, with the variations being most extreme in the antibiotic-free (control) lots. For example, the adsorption and the burst size varied 38 to 90% and from 10 to 91, respectively, for the different control trials.

Differences in adsorption averages between the different treatments were relatively slight, with the extremes being the terramycin (70.5%) and the penicillin (59.4%). The averaged variations were within ± 7% from the results for the antibiotic-free series.

The average burst sizes varied significantly between the different treatments, with the penicillin series having the highest value (84) and the terramycin series the lowest (16). The differences of the antibiotic series from the control series would indicate that the presence of penicillin accelerates the production of new phage particles, whereas terramycin and nisin tend to retard the production of new phage particles.

The latent period was prolonged by the presence of antibiotics with the average latent period (in minutes) for control, penicillin, terramycin, and nisin series being 16, 20, 24, and 23, respectively. Variations in latent periods in the individual trials were within 2 min.

The average rise period was the same for the control and the penicillin series (14 min), but was greater for the terramycin and nisin series, i.e., 22 and 21 min, respectively. This indicates that the rate of release of new phage is extended when either of the latter two antibiotics are present in the medium.

Since these data dealt with susceptible cells in the presence or absence of antibiotics, it seemed desirable to measure the response of resistant cells to penicillin. To ascertain what influence this factor will have on new phage production, a series of single-step growth curves were prepared in which penicillin-resistant *S. lactis* C10 host cells were used with phage c10. Results obtained from three trials are presented in Table 2.

Per cent adsorption and latent period for penicillin-resistant cells varied only slightly from values obtained when penicillin was added to susceptible *S. lactis* C10 cultures. However, the induction of penicillin resistance did influence the burst size. Penicillin-resistant cells had an average burst size of 38, as contrasted to 84 for susceptible cells in the presence of penicillin and 42 for the same cells in control broth. This would indicate that the ability of penicillin to stimulate increased phage production appears to be limited to the action of this antibiotic on susceptible cells. The rise period with resistant cells was 10 min, compared to 14 min with susceptible cells, either in the presence or absence of penicillin. The rapid release of newly formed phage particles may be related to the cell-wall fragility of the penicillin-resistant cells observed by Richards et al. (8).

DISCUSSION

The observed effect of the antibiotics on certain fundamental relationships between lactic streptococci and phage may be due to physical or chemical changes, or both, incited when antibiotic-susceptible cells come in contact with antibiotics. Several investigators (4, 8) have

TABLE 1

Single-step growth curve characteristics of Phage c_{10} with *S. lactis* C_{10} host cells in the presence of antibiotics

Experimental conditions	No. trials	Per cent adsorbed		Burst size		Latent period [a]		Rise period	
		Range	Avg	Range	Avg	Range	Avg	Range	Avg
						(min)			
Control	7	38-90	66.1	10- 91	42	15-17	16	10-18	14
Penicillin	4	42-75	59.4	34-145	84	19-21	20	10-20	14
Terramycin	5	58-83	70.5	5- 44	16	23-25	24	20-24	22
Nisin	4	53-66	60.5	11- 50	30	23-25	23	18-22	21

[a] Includes 5 min of adsorption.

TABLE 2

Single-step growth characteristics of Phage c_{10} with penicillin-resistant *S. lactis* C_{10} host cells

Per cent adsorbed		Burst size		Latent period [a]		Rise period	
Range	Avg	Range	Avg	Range	Avg	Range	Avg
				(min)			
49-63	57.6	37-40	38	17-21	19	8-12	10

[a] Includes 5 min of adsorption.

reported that exposure of susceptible cells to penicillin results in enlarged cells and changes in cell wall structure and permeability. Heden (7) was able to demonstrate that maximum burst size coincides with maximum cell volume and ribonucleic acid content. Differences in burst size between susceptible cells exposed to penicillin and those exposed to terramycin may be related to the ability of these antibiotics to regulate nucleic acid synthesis. Antibiotics have been reported to influence nucleic acid synthesis (4, 9). This would be important, since the nucleic acids of the host are precursors of newly formed phage particles (1). Exposure of susceptible cells to penicillin does not cause an immediate cessation of nucleic acid synthesis, despite the fact that cell division may have ceased (4). In the case of terramycin, Shahani (9) found that the nucleic acid content of terramycin-susceptible *S. lactis* cells was greater than that of resistant cells.

The observation that antibiotics will influence the latent period may be related to findings by Adams and Wassermann (2), who have demonstrated that antimetabolites, as well as the nutrition and genetic make-up of phage and host cells, will influence the latent period.

The magnitude of the burst size and, to a lesser extent, the latent period appear to be the factors accounting for Richards' et al. (8) observed differences in lysis-rate of lactic streptococci by phage in the presence of antibiotics.

REFERENCES

(1) Adams, M. H. Bacteriophages. Interscience. N. Y. 1959.

(2) Adams, M. H., and Wassermann, Frequency Distribution of Phage R in the One-Step Growth Experiment. ogy, 2: 96. 1956.

(3) American Public Health Associa Standard Methods for the Examinati Dairy Products. 11th ed. New York.

(4) Cooper, P. D. Site of Action of Radi cillin. Bacteriol. Revs., 20: 28. 195

(5) Deane, D. D., and Nelson, F. E. Mu Strain Bacteriophage Infections of mercial Lactic Starters. J. Dairy Sci 668. 1952.

(6) Delbruck, M., and Luria, S. E. Int ence Between Bacterial Viruses. I. ference Between Two Bacterial V Acting upon the Same Host and the anism of Virus Growth. Arch. Bio 1: 111. 1942.

(7) Heden, Carl-Goran. Studies of the tion of *E. coli* B with the Bacterio T2. Acta Pathol. Microbiol. Scand., 88. 1951.

(8) Richards, R. J., Kennedy, H. E., Gould, I. A. Antibiotic and Bacterio Interactions in Anti-Streptococcal Ac Bacteriol. Proc. Am. Soc. Microbiol. Ann. Meeting. p. 32 (A52). 1962.

(9) Shahani, K. M. Comparison of Acids and Other Phosphorus Com of Terramycin-Sensitive and -Re Strains of *Streptococcus lactis*. J. Sci., 42: 1659. 1959.

(10) Sobol, F. S. A Comparative Study Amino Acid Requirements of Terra Resistant and Terramycin-Sensitive of *Streptococcus lactis*. M.S. thesis Ohio State University. 1957.

FRACTIONATION OF THE STALE-FLAVOR COMPONENTS OF DRIED WHOLE MILK[1]

W. W. NAWAR,[2] S. H. LOMBARD,[3] H. E. T. DALL,[4] A. S. GANGULY,[5]
AND R. McL. WHITNEY
Department of Food Technology, University of Illinois, Urbana

SUMMARY

The stale flavor of dried whole milk has been shown to exist in two forms which can be separated by CCl_4-vapor distillation and Girard's-T extraction. One is nonvolatile under the conditions employed but can be extracted from the milk fat with Girard's-T reagent; the other is volatile and is not recovered from the Girard's-T extract after hydrolysis of the resulting hydrazones.

The Girard's-T extractible component is recovered more completely from the fat when lower concentrations of Girard's-T reagent are used, a behavior similar to aldehydes. It can be separated by paper or column chromatography into a stationary and a mobile component which contains the flavor. The R_f value of the mobile component is similar to that of the reaction product of heptaldehyde. It is readily decomposed by alkali or 2,4-dinitrophenylhydrazine in 4 M HCl. On treatment with 2,4-dinitrophenylhydrazine, at least four carbonyl compounds were obtained as decomposition products and tentatively identified by paper chromatography as acetaldehyde, propionaldehyde, acetone, and either 2-butanone or 2-pentanone.

The CCl_4-vapor distillates were found to contain at least seven carbonyl compounds, five of which have been identified as formaldehyde, acetaldehyde, propionaldehyde, acetone, and 2-penatone. The remaining two compounds possess the spectra and chromatographic characteristics of a saturated monocarbonyl compound and either an unsaturated dicarbonyl or hydroxycarbonyl compound. The stale-flavor component of this fraction appears to behave like a dicarbonyl compound with Girard's-T reagent.

The factors involved in the development of a stale flavor which appears early in the storage life of dried whole milk are complex and relatively obscure. Consequently, a considerable amount of research has been done to prevent or retard the development of this flavor by varying the methods of manufacture and storage (5, 15, 17, 20, 25, 28-30). More recently, attempts have been made to isolate and identify the causative substances and to secure information on the chemical changes involved (23, 32-34). Fundamental knowledge of this nature would prove very valuable in establishing processing procedures capable of preserving the fresh-milk flavor characteristics and controlling this flavor defect.

Whitney and Tracy (33, 34) fractionated reconstituted stale dry whole milk into cream, skimmilk, butter, buttermilk, butteroil, and butter serum and found that the stale-flavor component appeared to be distributed in proportion to the amount of fat present in each fraction. They concluded that the flavor is concentrated in the fat phase, but emphasized that this was not evidence as to the origin of the flavor. Steam distillation of the extracted butteroil yielded a detectable stale flavor when blended with fresh products in concentrations as low as 6.2 ppm (32).

The purpose of this study was to further fractionate the components responsible for the

Received for publication October 12, 1959.

[1] Taken in part from the theses of S. H. Lombard presented in February, 1955, W. W. Nawar presented in February, 1959, and A. S. Ganguly, presented in May, 1961, in partial fulfillment of the requirements for the degrees of Doctor of Philosophy.

[2] Present address: Department of Food Technology, University of Massachusetts, Amherst, Massachusetts.

[3] Present address: Division of Dairying, University of Pretoria, Pretoria, Republic of South Africa.

[4] Present address: A/S Lidano, Kalundborg, Denmark.

[5] Present address: Hindustan Lever, Ltd., Bombay, India.

flavor in dried whole milk and to secure further information as to their chemical and physical character.

EXPERIMENTAL METHODS

Manufacture and storage of dried whole milk. As needed throughout this study, whole milk was condensed and dried to 2.2 to 3.0% moisture in a pilot-size experimental spray dryer. The powders were held at room temperature in air in sealed containers until they developed a typically stale flavor, then were stored at −14 F to insure a continuous supply of stale whole milk powder.

Preparation of stale milk fat. Milk fat was extracted from the stale dried whole milk immediately before each experiment by the procedure of Whitney and Tracy (34). While modifications in this procedure were introduced at various times in the study, in order to handle larger amounts of powder and speed the extraction process, it consisted essentially of freeing the fat for extraction by agitating the dried whole milk with purified 95% ethanol[6] containing enough additional water to increase the moisture content of the powder to 8%, extracting the fat with purified low-boiling petroleum ether,[7] removing the bulk of the alcohol from the extract by agitation with water, and finally removing all but traces of the solvents from the fat by aspiration at 40 C. All modifications of this procedure used were found to yield a fat containing the stale flavor.

Solvent removal and flavor evaluation. At each stage in the fractionation procedure, the flavor of the fraction in question was evaluated. Its CCl_4 solution was added to a weighed amount of fresh milk fat and the solvent removed by aspiration for 1 hr, followed by the application of high vacuum (0.001 mm Hg) for 3.5 hr with the aid of a high-vacuum pump. This milk fat was then homogenized into fresh skimmilk to the composition of whole milk (4% fat). Whenever a qualitative examination of the flavor was desired, the samples were submitted to the judging panel in the form of the triangle test described by Lockhart (21), with the fresh milk fat homogenized into skimmilk as a control. Where a quantitative measurement of the flavor was required, the threshold concentration of the stale sample was established by using the technique described b Whitney and Tracy (33).

Infrared analysis of fractions. The infrare adsorption spectra of the various fraction were obtained in CCl_4 solution dried over an hydrous Na_2SO_4 with a Perkin-Elmer Model 2 Infrared Recording Spectrophotometer wit NaCl cells, 0.5 or 1.0 mm thick.

CCl_4-vapor distillation. The volatile components of the milk fat were removed by th CCl_4-vapor distillation method of Chang an Kummerow (4). The apparatus, similar t that designed by Bailey and Feuge (1), consisted of a boiler, a retort, and two cold finger refrigerated by means of liquid nitrogen. CC in the boiler and stale milk fat in the retor were both frozen before distillation, and th system was evacuated with the aid of a high vacuum pump (0.001 mm Hg). The temperature of the milk fat was then raised to 40 C and that of the CCl_4 to room temperatur Under high vacuum, the CCl_4 vapor passe through a sintered-glass sparger submerge in the milk fat and, along with the volati materials present, condensed on the two col fingers, while any uncondensable gases passe by a McLeod gauge and were finally remove by the mercury-diffusion pump. Two hour after the fat had completely melted, the pum was shut off, the system opened, the liqui nitrogen replaced with warm water (40 C and the condensates collected by washing th fingers with fresh CCl_4.

Recovery of the volatiles from the CCl_4-vap distillate by preparatory gas chromatograph Twelve grams of trilaurin (Eastman) and 48 of 40-60 mesh firebrick were added to th CCl_4-vapor distillate in a rotary evaporato and the solvent removed by aspiration at 50 C The dry material was then packed in the preparatory gas-chromatographic column of Ganguly (11). The column was heated to 100 C helium gas was passed through it at the rate 60 ml/min for 5 hr, and the volatiles were collected in a trap cooled in liquid nitrogen.

Girard's-T extraction. The Girard's-T-extractible components of milk fat were obtain by shaking it with methanol or ethanol sol tions of Girard's-T reagent (trimethylaceth drazide ammonium chloride) (12, 13), usual 2 mg of reagent per gram of fat for 1.75 at 40 C. The reaction products were separat from the fat by washing with an equal volu of water, and traces of fat removed from th extract by agitation with an equal volume purified petroleum ether, followed by centri gation to break any emulsion formed. The water extract was then acidified with 5 M HCl to a final concentration of 0.5 M, and the resulting mixture shaken at room temperature for 1 hr to hydrolyze the Girard's-T hydrazones. The liberated compounds were then extracted in CCl_4, with agitation followed by centrifugation.

---

[6] The ethanol was purified by refluxing ethanol with 10 g of 2,4-dinitrophenylhydrazine per liter for 4 hr, then distilling twice. Such a procedure removes the bulk of the carbonyl impurities.

[7] Petroleum ether (B.P. 40 C) was washed four times with distilled water, refluxed with 5 g of 2,4-dinitrophenylhydrazine and 1 g of trichloroacetic acid per liter for 4 hr, then distilled to secure a solvent free of most of the carbonyl compounds.

Chromatography of the Girard's-T hydrazones. For the characterization of the components of the Girard's-T extract the paper chromatographic procedure of Zaffaroni et al. (35) was adopted. The water extract of the hydrazones was concentrated ten to one in a rotary evaporator at 40 C and 0.2 to 0.4 ml of it applied to the Whatman No. 1 filter paper strip. After development of the chromatogram, the components were located by treating the strip with iodoplatinate solution prepared by adding 5 ml of a 5% solution of $PtCl_4$ (C.P.) in 1 M HCl and 65 ml of a 10% solution of KI (C.P.) to 100 ml of distilled water.

The chromatographic fractionation of the Girard's-T extract was accomplished by column chromatography. The column employed consisted of a pyrex-glass tube 25.4 cm high and 3.2 cm in diameter packed (to a depth of 4 cm) with standard grade Whatman cellulose powder saturated with water equilibrated against butanol. The sample applied to the column was prepared by adding 2.5 g of cellulose powder to the water extract containing the Girard's-T hydrazones and removing the water in a rotary evaporator at 40 C. After replacing the excess water in the column with butanol which had previously been equilibrated against water, the dried sample was placed on top of the column and kept in place by a cardboard filter disc. The mobile phase was then passed through the column at a rate of 10 ml per hour until 26 ml were collected (the volume of eluate was calculated to be sufficient to remove the mobile component from the column). The eluate was shaken with equal volumes of water and purified petroleum ether, and the carbonyl compounds recovered by hydrolysis and extraction with CCl_4 in the usual manner.

Preparation and chromatography of the 2,4-dinitrophenylhydrazones. To prepare the 2,4-dinitrophenylhydrazones of the carbonyl compounds present in the various fractions obtained, the fraction was shaken with an appropriate volume of a saturated solution of 2,4-dinitrophenylhydrazine in 4 M HCl for 18 hr at room temperature. The hydrazones were extracted with an equal volume of CCl_4, and the CCl_4 layer concentrated to a volume of 1 ml.

Paper chromatograms of the 2,4-dinitrophenylhydrazones were prepared either by the method of Clements and Deatherage (6) or Huelin (16).

Identification and characterization of 2,4-dinitrophenylhydrazones. Tentative identifications of the 2,4-dinitrophenylhydrazones were secured by comparison of their R_f values with those of known carbonyl compounds reported in the literature. More definite identification was obtained by the comparison of their R_f values with those of known carbonyl derivatives upon the same chromatogram, and by comparison of their absorption spectra in ethanol and alkaline ethanol with those of the known compounds. The hydrazones were eluted separately from the chromatogram with a minimum amount of purified 95% ethanol and their spectra determined over the wave length range of 325-625 mμ on a Cary Model 11 Recording Spectrophotometer. The solutions were then made alkaline by mixing them in the ratio of two to one with alcoholic NaOH prepared by the method of Jones et al. (18), and the spectra obtained immediately to avoid fading.

RESULTS AND DISCUSSION

While it is generally accepted that flavor of a dairy product is the integrated combination of flavor sensations due to all of the flavor components in the product, there is some disagreement as to the proper approach to the problem of an off-flavor in the products. One view is based upon the concept that the off-flavor is also the result of the combination of flavor sensations. In contrast, those investigators who hold the other view believe that the particular off-flavor sensation can be singled out from the other sensations present and that this sensation is due to a certain steric configuration which, while it may be present in a number of different compounds, must be present before the flavor is detected. This view is admirably presented by Moncrieff (22), and is supported by evidence in the literature, in that all chemically identified flavors have been attributed to specific compounds or configurations such as the coconut flavor of δ-decalactone (19), the 2-enals of C_8 and C_9 responsible for the oxidized flavor (8), the sunlight flavor of methional (24), and the metallic flavor of vinyl amyl ketone (27). The approach employed in this study is based upon the second opinion and, therefore, the emphasis is placed upon the fractionation and isolation of only those components which, when reconstituted to whole milk composition, yield a detectable stale flavor.

Such an approach to the identification of the components responsible for an off-flavor is complicated by several factors. Off-flavors may often be produced by traces of materials which escape detection by the analytical techniques employed. Fractions containing the flavor usually consist of a mixture of compounds, the majority of which are not responsible for the off-flavor. In some cases, the flavor components are unstable and undergo changes during the fractionation procedure. Throughout this study, experiments were conducted in the shortest time possible and care taken to avoid exposure to light, heat, and air, wherever possible. The organoleptic evaluation of the flavor as a tool for following the flavor components has its uncertainties. The term stale flavor is apparently not uniformly defined. However, in this study a product was reported as stale only if it possessed a characteristic organoleptic sensation agreed upon by the judging panel to be the major flavor sensation of stale dried whole milk. Variations in the threshold responses of the judges also required that the various fractionation procedures be repeated numerous times, to confirm the behavior of the stale-flavor components. Therefore, the methodology reported in this paper is limited to the successful fractionation and characterization procedures, and the results of the numerous fractionation experiments are summarized in the schematic diagram, Table 1.

Fractionation of the stale-flavor components by CCl_4-vapor distillation and Girard's-T extraction. Throughout this study it was observed that, while at certain times in the storage life of a dried whole milk, readily detectable quantities of the stale-flavor components could be obtained from the stale milk fat by the Girard's-T-extraction method, at other times, with the same procedure, it was impossible to do so. At such times, a shift to the CCl_4-vapor distillation technique resulted in appreciable quantities of the flavor components. Occasionally, apparently in the transition periods, the flavor could be detected in both fractions. Such a situation is represented in the schematic diagram, Table 1. From this, it can be seen that at these times, when the CCl_4-vapor distillation is performed first, there remains in the fat some stale-flavor component which is extractible by Girard's-T reagent. However, when the Girard's-T extraction is performed first, only occasionally can a stale-flavor component be removed from the fat by CCl_4-vapor distillation. Since it is well known that the fractionation procedures when applied to trace materials are rarely complete, it was first thought that these results were due t incomplete removal of the stale-flavor components by the method employed. However the repeated observation that the stale-flavo component could be secured from stale milk fa by one method or the other, but rarely by bot at a given time in the storage life of the powder, necessitated a reinterpretation of the dat and further experimentation.

It was found that when the Girard's-T ex tract of stale milk fat in CCl_4 was subjecte to CCl_4-vapor distillation, in the same manne as the stale milk fat except that the distillatio was continued until all the CCl_4 was remove from the retort, that the CCl_4-vapor distillat did not contain the stale-flavor, while the residue in the retort was considered to be stale b the judging panel. Therefore, the Girard's-T extractible stale-flavor component was not volatile under the condition used for separating th volatile component from stale milk fat.

Similarly, it was found that, when the CCl vapor distillate, which was shown to contai a stale-flavor component, was added to fres fat, the CCl_4 removed in the same manner a for flavor evaluation, and the residual fat subjected to Girard's-T extraction in the usu manner, the Girard's-T extract was not considered stale by the judging panel. Therefor stale-flavor component of the CCl_4-vapor distillate was not recovered from fresh fat b the Girard's-T extraction procedure.

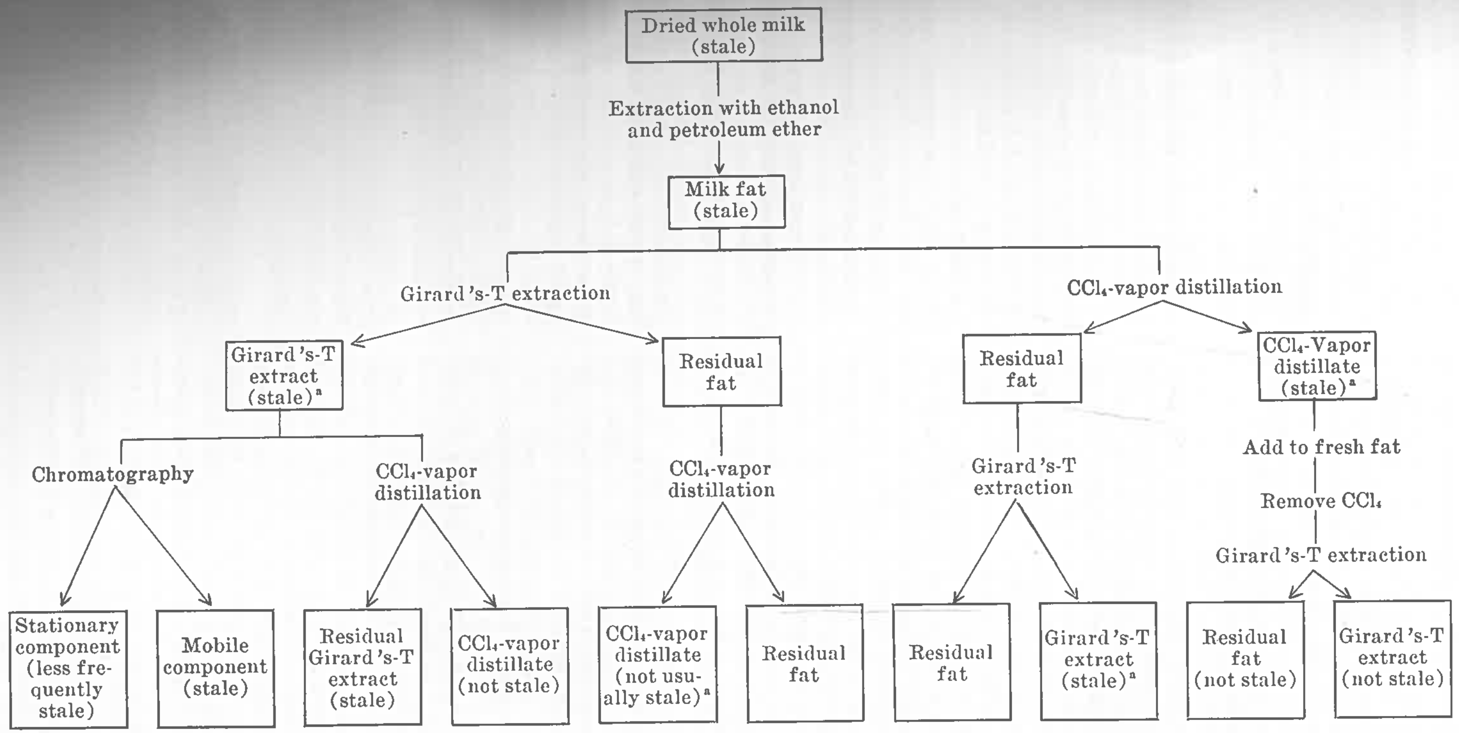
It appears that there are at least two different compounds capable of causing the stale-flavor sensation and that the quantity of the components present in the dried whole mil varies with the time and conditions of storag of the dried whole milk.

While these studies were not designed t investigate the effect of storage upon the tw forms of the stale flavor, the available eviden suggests that this flavor occurs first as th Girard's-T extractible component.

Characterization of the Girard's-T-extractib stale-flavor component. The infrared absorption spectra of the Girard's-T-extractible fraction obtained from stale milk fat indicated th presence of two different carbonyl group one in the ester-lactone region (1,745 cm and the other in the ketone-aldehyde regio ($1{,}712\ cm^{-1}$) (3). Attempts to further fractionate the material by alkaline extractio however, resulted in the destruction or loss the stale flavor and the disappearance of t $1{,}712\ cm^{-1}$ absorption maximum from both t alkaline extract and the residual Girard's extract. The absorption maximum at 1,745 cm was not observed in the alkaline extract,

TABLE 1

Schematic diagram of fractionation of the stale-flavor components of dried whole milk


Dried whole milk (stale)
Extraction with ethanol and petroleum ether
Milk fat (stale)
Girard's-T extraction
CCl₄-vapor distillation
Girard's-T extract (stale)ᵃ
Residual fat
Residual fat
CCl₄-Vapor distillate (stale)ᵃ
Chromatography
CCl₄-vapor distillation
CCl₄-vapor distillation
Girard's-T extraction
Add to fresh fat
Remove CCl₄
Girard's-T extraction
Stationary component (less frequently stale)
Mobile component (stale)
Residual Girard's-T extract (stale)
CCl₄-vapor distillate (not stale)
CCl₄-vapor distillate (not usually stale)ᵃ
Residual fat
Residual fat
Girard's-T extract (stale)ᵃ
Residual fat (not stale)
Girard's-T extract (not stale)

[a] While usually the flavor is found predominantly in one or the other of these two forms, it has been observed to occur in both forms simultaneously.

remained in the residual. Another approach to the further fractionation of the Girard's-T extract by the use of sodium bisulfite also resulted in the loss of the stale flavor and the disappearance of the 1,712 cm^{-1} absorption maximum. Since alkaline conditions were necessary for the hydrolysis of the bisulfite-addition products, these results would suggest that the stale-flavor component in the Girard's-T extract is an aldehyde or methyl ketone and is destroyed in the alkaline pH range.

The work of Ganguly (10) on the extraction of various known carbonyl compounds from milk fat indicated that their recovery was greatly influenced by the concentration of Girard's-T reagent and was dependent upon the type of carbonyl compound. A study of the effect of concentration of Girard's-T reagent upon the recovery of this stale-flavor component from stale milk fat indicated that, at a concentration of 2 mg of reagent per gram of fat, the CCl$_4$ extract of the hydrolysis products of the reaction contained a considerable amount of the stale flavor, while at higher concentrations, 200 mg and 400 mg of reagent per gram of fat, no detectable amounts of stale flavor were observed. Infrared absorption spectra of the extracts showed a progressive decrease in the absorption at 1,745 cm^{-1} and 1,635 cm^{-1} as the concentration of reagent was increased and the absorption at 1,712 cm^{-1} became more apparent. This observation of a greater recovery of the stale-flavor component at lower concentrations of Girard's-T reagent suggests that it is an aldehyde, since Ganguly (10) observed a similar behavior in the case of aldehydes, whereas monoketones are more completely recovered at higher concentrations of the reagent.

To tentatively establish the number of components present in the Girard's-T extract, and to determine whether chromatography could serve as a means for the further fractionation of this extract, paper chromatograms of the Girard's-T hydrazones present were obtained by the method of Zaffaroni et al. (35). These chromatograms indicate the presence of two components, one stationary and the other mobile, with an R_f value of 0.72-0.80. The mobile component developed a pinkish-brown color when stained with iodoplatinate solution. These properties are similar to those of the Girard's-T hydrazone of heptaldehyde (10). Flavor evaluation of authentic heptaldehyde (Eastman), however, indicated that it was not responsible for the stale flavor.

To determine which component of the Girard's-T extract was responsible for the stale flavor, these components were separated o paper, modifying the procedure so as to obtain adequate amounts of the two componen for flavor evaluation. While sometimes bot the stationary and the mobile components we considered to be stale by the judging pan the mobile fraction was judged stale more fr quently than the stationary. The presence the stale flavor in the stationary componen when it was detected, may have been due the entrapment of the mobile component the stationary spots due to the presence of precipitate in the concentrated water extra or to a stale-flavor component less readily covered upon hydrolysis of the hydrazones.

Since paper chromatography is not a co venient way to separate large quantities of t mobile component of the Girard's-T hydrazon from the rest of the material, the column chr matographic technique was developed for th purpose and proved to be satisfactory.

The mobile component isolated in this ma ner was further characterized by paper chr matography of its 2,4-dinitrophenylhydrazon by the method of Clements and Deatherage ((Table 2). Since three of the components observed were also present in the chromatogra of the CCl$_4$ used for extraction, they may n all have originated from the mobile componen A comparison of the R_f values and color these spots with data obtained by Clements a Deatherage (6) and Tuckey and Colmey (3 for known carbonyl compounds indicated th Spots 2, 5, 6, and 7 could have been due to t presence on the reaction mixture of acetald hyde, propionaldehyde, acetone, and 2-but none or 2-pentanone, respectively. The pr ence of all of these compounds in stale dr whole milk has been subsequently conclusive confirmed by Parks et al. (23). However, no

TABLE 2

Paper chromatography of the 2,4-dinitrophen hydrazones of the Girard's-T-extractible mobile stale-flavor component

R_f values				Alkaline color
CCl$_4$[a]	Stale-flavor component			(10% KO
100λ	50λ	75λ	100λ	
0.39	0.39	0.39	0.40	Yellow
....	0.42	0.44	0.44	Tan
0.46	0.46	0.47	0.48	Tan
0.52	0.52	0.52	0.53	Tan
......	....	0.57	0.57	Tan
......	0.61	0.61	0.61	Tan
			0.67	Tan

[a] CCl$_4$ used to extract 2,4-dinitrophenylhy zones from reaction mixture.

of them could be shown to be responsible for the stale flavor. Two possible explanations exist for the presence of more than one 2,4-dinitrophenylhydrazone in the reaction mixture obtained from a single mobile Girard's-T hydrazone. Either the single mobile spot was not resolved and contains more than one hydrazone, or the reaction with 2,4-dinitrophenylhydrazine caused a breakdown of the mobile component. The tentative identification of the 2,4-dinitrophenylhydrazones indicated makes it very doubtful that the mobile Girard's-T hydrazone was an unresolved mixture because the R_f values of these carbonyl compounds in their Girard's-P-hydrazone form are considerably lower than 0.72-0.80 (26) and Girard's-P hydrazones have similar R_f values to Girard's-T hydrazones (26). Therefore, it is apparent that the mobile component was broken down by the treatment with 2,4-dinitrophenylhydrazine in 4 M HCl. This was further substantiated by the absence of any stale odor when the carbonyl compounds were released from the 2,4-dinitrophenylhydrazones by H_2SO_4 according to the method of Bassette and Day (2).

Characterization of the CCl_4-vapor distillate of stale milk fat. Infrared absorption spectra of the CCl_4-vapor distillate of stale milk fat indicated the presence of carbonyl groups with absorption maxima at 1,745 cm^{-1} and 1,712 cm^{-1}. As indicated in the schematic diagram, treatment with Girard's-T reagent of the fresh milk fat containing the components of the CCl_4-vapor distillate resulted in the absence of the stale flavor in both the carbonyl compounds recovered by hydrolysis of the Girard's-T extract and the residual milk fat. Therefore, the stale-flavor component in the CCl_4-vapor distillate appears to be a carbonyl compound which is removed from the milk fat by Girard's-T reagent but not recovered upon hydrolysis of the Girard's-T hydrazones. The work of Ganguly (10) indicates that dicarbonyl compounds, while removed from the milk fat almost completely by Girard's-T extraction, are not appreciably recovered from the hydrolysate due to incomplete hydrolysis.

To further characterize this stale-flavor component by treatment with 2,4-dinitrophenylhydrazine, it was desirable to secure this volatile fraction free of solvent, since this reaction may not be sufficiently complete in a biphasic system to obtain definite evidence regarding the carbonyl nature of this component. Efforts to remove it from the stale milk fat by vacuum distillation or nitrogen entrainment failed to do so, even though subsequent vapor distillation with CCl_4 demonstrated that the stale flavor was present in this form. The recovery of the stale flavor from the CCl_4-vapor distillate free of CCl_4 was finally accomplished by the preparatory gas-chromatographic procedure described in the section on methods. The stale flavor was shown to be in the eluate of the gas-chromatographic column and to be recovered in the liquid-nitrogen trap.

When the material in the trap was treated with 2,4-dinitrophenylhydrazine and extracted with CCl_4 in the usual manner, and the volatile constituents removed from the extract by CCl_4-vapor distillation, the stale flavor was absent from the distillate. Paper chromatography of the hydrazones by Huelin's method (16) indicated the presence of seven compounds (Table 3). Five of these compounds could be identified by the comparison of their R_f values and absorption spectra with that of known compounds and were found to be formaldehyde, acetaldehyde, propionaldehyde, acetone, and 2-pentanone. None of these compounds proved to be responsible for the stale flavor. Although the R_f value of Spot no. 6 in the chromatogram suggests the presence of butyraldehyde, comparison of its absorption spectra with that of the 2,4-dinitrophenylhydrazone of butyraldehyde did not indicate sufficient agreement for identification. While the slowest-moving component in the chromatogram possessed an R_f value similar to the derivatives of diacetyl, acetoin, hydroxyacetone, and glyoxal, its absorption spectra differed markedly from those of the authentic compounds, but retained the characteristics of a hydroxycarbonyl or dicarbonyl compound. The apparent shift (to higher wave lengths) both in alcoholic and alkaline media suggests the possibility of unsaturation in the molecule, since this effect has been noted in monocarbonyl compounds (7-9, 14, 18). If this compound is responsible for the stale flavor of the CCl_4-vapor distillate, this would explain the disappearance of the stale flavor from the milk fat containing the components of the CCl_4-vapor distillate upon treatment with Girard's-T extract and its absence from the hydrolyzed Girard's-T extract, since this is a characteristic of dicarbonyl compounds (10). It would also explain the usual absence of the stale flavor from the CCl_4-vapor distillate of the residual fat from a Girard's-T extract of stale milk fat.

CONCLUSIONS

This study, therefore, indicates that the stale-flavor sensation observed in dried whole milk may be present in either or both of two chemical forms. One form can be recovered from

TABLE 3

Comparison of the chromatographic and spectral characteristics of the 2,4-dinitrophenylhydrazones from the CCl_4-vapor distillate from stale milk fat with those of known carbonyl compounds

CCl_4-vapor distillate				Authentic compounds			
		λ max (mμ)				λ max (mμ)	
Spot No.	R_f	Ethanol	Alkaline ethanol	Compound	R_f	Ethanol	Alkaline ethanol
1	0.04	361	367,$\underline{539}$[a]	Diacetyl	0.07	350	500
				Acetoin	0.07	350	500
				Hydroxyacetone	0.03	354	450,$\underline{512}$
				Glyoxal	0.03	346	400,$\underline{494}$
2	0.18	337	$\underline{439}$,520	Formaldehyde	0.20	340	$\underline{440}$,520
3	0.30	358	$\underline{435}$,525	Acetaldehyde	0.29	358	$\underline{432}$,525
4	0.37	356	$\underline{435}$,525	Propionaldehyde	0.39	357	$\underline{433}$,525
5	0.43	360	$\underline{434}$,525	Acetone	0.38	361	$\underline{432}$,525
6	0.52	360	$\underline{440}$,525	Butyraldehyde	0.49	347	$\underline{430}$,517
7	0.64	360	$\underline{435}$,525	2-Pentanone	0.60	361	$\underline{434}$,525

[a] Underlined λ max represents major peak.

stale milk fat by Girard's-T extraction, but is not removed by CCl_4-vapor distillation. It appears to be an aldehyde the Girard's-T hydrazone of which has an R_f value similar to that of heptaldehyde and to be readily decomposed by treatment with alkali or 2,4-dinitrophenylhydrazine in 4 M HCl. The other form can be volatilized from stale milk fat by CCl_4-vapor distillation and reacts with Girard's-T reagent, but is not readily recovered from the reaction product by hydrolysis. It reacts with 2,4-dinitrophenylhydrazine and may be unsaturated dicarbonyl or hydroxycarbonyl compound.

ACKNOWLEDGMENTS

This paper reports in part research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and has been assigned 1058 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Department of the Army.

The authors wish to express their appreciation to the other members of the staff of the Division of Dairy Technology for serving as judges and to Dr. J. H. Hetrick and the Dean Milk Company, Rockford, Illinois, for preparing some of the dried whole milk used in this study. The services of the Spectrographic Laboratory of the University of Illinois for the infrared absorption spectra are greatly appreciated.

REFERENCES

(1) Bailey, A. D., and Feuge, R. O. Laboratory Deodorizer for Fats and Oils. Ind. Eng. Chem., Anal. Ed., 15: 280. 1943.

(2) Bassette, R., and Day, E. A. Regenerati
of Carbonyl Compounds from 2,4-Dinit
phenylhydrazones with Sulfuric Aci
J. Am. Oil Chemists' Soc., 37: 482. 196

(3) Bellamy, L. J. The Infra-red Spectra
Complex Molecules. Methuen and Co., Lt
London. p. 114. 1954.

(4) Chang, S. S., and Kummerow, F. A. D
partment of Food Technology, Universi
of Illinois, Urbana. Personal communic
tion. 1955.

(5) Christensen, L. J., Decker, C. S., A
Ashworth, U. S. The Keeping Quality
Whole Milk Powder. I. The Effect
Preheat Temperature of the Milk on
Development of Rancid, Oxidized and Sta
Flavors with Different Storage Conditio
J. Dairy Sci., 34: 404. 1951.

(6) Clements, R. L., and Deatherage, F.
A Chromatographic Study of Some of
Compounds in Roasted Coffee. Food
search, 22: 222. 1957.

(7) Forss, D. A., Dunstone, E. A., A
Stark, W. Fishy Flavour in Dairy Pro
ucts. II. The Volatile Compounds As
ciated with Fishy Flavour in Butterf
J. Dairy Research, 27: 211. 1960.

(8) Forss, D. A., Pont, E. G., and Stark,
The Volatile Compounds Associated
Oxidized Flavour in Skim Milk. J. Da
Research, 22: 91. 1955.

(9) Gaddis, A. M., Ellis, R., and Currie, G.
Carbonyls in Oxidizing Fat. I. Separa
of Steam Volatile Monocarbonyls
Classes. Food Research, 24: 283. 1959.

(10) Ganguly, A. S. The Use of Girard'
Reagent in the Extraction and Charac
zation of Carbonyl Compounds. M.S. th
University of Illinois, Urbana. 1959.

(11) Ganguly, A. S. Studies on the Nature of the Stale-Flavor Components in Dried Whole Milk. Ph.D. thesis, University of Illinois, Urbana. 1961.

(12) Girard, A., and Sandulesco, G. Sur une novelle Serie de Reactifs du Groupe Carbonyle, leur Utilization a l'Extraction des Substances cetoniques et a la Caracterisation microchimique des Aldehydes et Cetones. Helv. Chim. Acta, 19: 1095. 1936.

(13) Hammond, E. G., and Bird, E. W. The Isolation and Identification of the Carbonyl Compounds Resulting from the Oxidation of Butteroil. J. Dairy Sci., 38: 593. 1955.

(14) Harper, W. J., and Bassett, E. W. Isolation and Identification of Acidic and Neutral Carbonyl Compounds in Different Varieties of Cheese. J. Dairy Sci., 41: 1206. 1958.

(15) Hetrick, J. H., and Tracy, P. H. Some Observations of the Keeping Quality of Dry Whole Milk Stored at Room Temperature. J. Dairy Sci., 28: 387. 1945.

(16) Huelin, F. E. Volatile Products of Apples. III. Identification of Aldehydes and Ketones. Australian J. Sci. Research, B. 5: 328. 1952.

(17) Hunziker, O. F. Condensed Milk and Dried Milk Products. 6th ed. Published by the author, La Grange, Illinois. p. 442. 1946.

(18) Jones, A. L., Holmes, C. J., and Seligman, R. B. Spectrophotometric Studies of Some 2,4-Dinitrophenylhydrazones. Anal. Chem., 28: 191. 1956.

(19) Keeney, P. G., and Patton, S. Isolation and Identification of the δ-Decalactone as the Compound Responsible for the Coconut-like Flavor of Butteroil. J. Dairy Sci., 38: 592. 1955.

(20) Lea, C. H., Moran, T., and Smith, J. A. B. The Gas-Packing and Storage of Milk Powder. J. Dairy Research, 13: 162. 1943.

(21) Lockhart, E. E. Binominal System and Organoleptic Analysis. Food Technol., 5: 428. 1951.

(22) Moncrieff, R. W. The Chemical Senses. John Wiley & Sons, New York. pp. 173-235. 1946.

(23) Parks, O. W., Wong, N. P., and Patton, S. Some Volatile Carbonyls from Stale Dry Whole Milk. J. Dairy Sci., 42: 912. 1959.

(24) Patton, S. The Mechanism of Sunlight Flavor Formation in Milk with Special Reference to Methionine and Riboflavin. J. Dairy Sci., 37: 446. 1954.

(25) Remaley, R. J. Fat Problems in Dairy Products. (Manual) QMC 1717. 19-21. 1945.

(26) Seligman, R. B., Edmonds, M. G., O'Keefe, A. E., and Lee, L. A. Paper Chromatography of Aldehydes and Ketones as Girard Derivatives. Chem. and Ind. (London), p. 1195. 1954.

(27) Stark, W., and Forss, D. A. A Compound Responsible for the Metallic Flavor in Dairy Products. I. Isolation and Identification. J. Dairy Research, 29: 173. 1962.

(28) Tarassuk, N. P., and Jack, E. L. Biochemical Changes in Whole Milk Powder and Ice Cream Powder During Storage. J. Dairy Sci., 29: 486. 1946.

(29) Tillmans, J., and Strohecker, R. Uber Krause-Milchpulver. Z. Untersuch. Nahr. Genussm., 47: 377. 1924.

(30) Tracy, P. H., and Wilster, G. H. Prevention of the Development of Stale Flavor in Dry Whole Milk During Storage. Termination Rept. QMC Food and Container Research Inst. for the Armed Forces. File BD-3, Rept. no. 4.

(31) Tuckey, S. L., and Colmey, J. C. Department of Food Technology, University of Illinois, Urbana. Personal communication. 1958.

(32) Whitney, R. McL., Paulson, K., and Tracy, P. H. Stale-Flavor Components from Stale Butteroil. III. The Steam Distillation of Stale-Flavor Components from Stale Butteroil. J. Dairy Sci., 33: 281. 1950.

(33) Whitney, R. McL., and Tracy, P. H. Stale-Flavor Components in Dried Whole Milk. I. The Distribution of Stale Flavor Between Fractions of Reconstituted Stale Whole Milk Powder. J. Dairy Sci., 32: 383. 1949.

(34) Whitney, R. McL., and Tracy, P. H. Stale-Flavor Components in Dried Whole Milk. II. The Extraction of Stale Butteroil from Stale Dried Whole Milk by Organic Solvents. J. Dairy Sci., 33: 50. 1950.

(35) Zaffaroni, A., Burton, R. B., and Keutmann, E. H. The Application of Paper Partition Chromatography to Steroid Analysis. J. Biol. Chem., 177: 109. 1949.

VARIATIONS IN PER CENT OF PROTEIN, MILK FAT, AND SOLIDS-NOT-FAT BETWEEN MILKINGS AND DURING THE MILKING PROCESS[1]

H. C. GILMORE[2] AND S. N. GAUNT

Department of Dairy and Animal Science, University of Massachusetts, Amherst

SUMMARY

A total of 1,290 individual evening and morning milk samples in 11 herds of three breeds was tested for protein, solids-not-fat, and milk fat. Morning sample means were lower than evening samples for all components, milk fat 0.73% lower, solids-not-fat 0.11%, and protein 0.03%; whereas, milk weights were 4.21 lb higher. These differences and those between herds, herds by time of day and breed were all statistically highly significant. The differences between milking intervals of 12-12 and 10-14 hr were highly significant for milk fat and significantly different for solids-not-fat.

Ten cows of each of four breeds were sampled at four stages of the milking process in the evening and morning. Protein and solids-not-fat percentages tended to drop as the milking process progressed, but the milk fat percentage increased markedly. The differences for solids-not-fat and fat were highly significant.

Aliquot samples of evening and morning milk are not needed for protein testing, but they would be needed for solids-not-fat testing based on the milk fat test.

Wide variations in the fat content of milk from individual cows have been recognized for many years. Anderson (1), as early as 1914, reported that milk fat varied at least 2.0 percentage points for 27.6% of 1,000 Holstein and Jersey cows during seven-day tests for Advanced Registry programs where individual milkings were tested. Espe and Smith (4) reported wide variations in milk composition, but some of these conclusions were based on limited data. Schmidt and Trimberger (12) found milk fat percentage to be higher in lactations for cows milked at wide intervals, 10-14 or 8-16 hr between milkings compared to equal intervals.

Anderson (1), Fosgate (5), Nicholson et al. (11), and Thompson et al. (14) reported that milk produced by dairy cows at the evening milking was significantly higher in milk fat content than the morning milk. In contrast to these reports, Specht et al. (13) found no significant difference in milk fat tests for evening and morning milk; whereas, Lolkem and Have (10) found a lower percentage o milk fat in the evening milk.

Specht et al. (13), Nicholson et al. (11 and Thompson et al. (14) reported no significant differences between the evening an morning solids-not-fat content of milk. Kliesc et al. (9) found slightly higher protein pe centage in the individual cow's milk in th evening samples. Specht et al. (13), reporte no significant difference in protein content o evening and morning milk.

Increases in milk fat percentages during th milking process have been reported by Esp (4), Fosgate (5), Gardner et al. (6), an Borsuk and Saks (2). Johansson et al. (8 in a study of milk from 1,836 individual qua ters, reported the average difference betwe first- and last-drawn milk to be 8% fat.

Fosgate (5) found a significant change i solids-not-fat percentage of milk in Jers but not Holstein cows as milking progresse Czako (3) reported no change in solids-not-f or protein percentage during the milking pr ess. Gardner et al. (6) found higher solids-n fat percentages in the middle portion of mi than in the fore milk or strippings when pressed on a fat-free basis.

In view of the limited work and the vari results reported on milk composition betw

Received for publication March 7, 1963.

[1] Contribution from the Massachusetts Experiment Station, University of Massachusetts, Amherst, Mass.

[2] Present address: Department of Dairy Science, Pennsylvania State University, University Park, Pennsylvania.

evening and morning milkings and during the milking process, a study was initiated to determine (1) the differences in milk composition between evening and morning milk and to see if these differences were affected by different milking intervals and (2) what differences occur in milk as milking progresses.

EXPERIMENTAL PROCEDURE

Two-ounce samples of milk were collected in sample jars containing the Golding plastic beads. The samples were cooled immediately and kept under refrigeration until delivered to the laboratory. No preservatives were added to the samples. Samples taken in the evening and morning were tested the day the morning samples were collected.

All samples were tested for milk fat by the Babcock test, for solids-not-fat by the Golding plastic bead method, and for protein by the Orange G dye binding procedure.

To determine differences in milk composition due to time and interval of milking, milk samples from approximately 100 cows of each of three breeds (Guernsey, Holstein, and Jersey) were obtained from two groups of cows. One group was milked at wide intervals, 10-14 hr, between milkings and the other at approximately equal intervals. Eleven herds, some of which had more than one breed, were represented. Samples were taken during the winter months and in the summer from the same herds. All cows in milk at each time were sampled.

Samples of milk from the herds of the cooperating dairymen were taken by the Dairy Herd Improvement Association supervisors who were cooperating in the Genetic Composition Milk Project at the University of Massachusetts.

To determine the effect of the stage of the milking process on the percentage of milk fat, protein, and solids-not-fat, samples of evening and morning milk were taken at four stages of the milking process. The first, second, and third minutes of milking and any milk produced after 3 min were used for the four portions of milk to be sampled.

A method for collection of the samples was devised that did not interrupt the milking process. Flat lids with two nipples of a size that would fit milk hoses were fitted to two 10-qt milk cans. They were assembled in a wooden rack and three-way valves were connected to each can. The milk hose from the claw assembly was connected to one three-way valve and the milkline hose was connected to the other valve. In this way, the milk could be collected in one can and then the other without interrupting the milking process.

Samples of milk were collected during the evening and morning milkings from ten cows of each of the four breeds (Ayrshire, Guernsey, Holstein, and Jersey) maintained by the University of Massachusetts. Some preference was given to selecting the higher-producing cows, since any differences which might be present would be more important in the higher-producing cows. However, because of the relatively small numbers of cows in the University herd, selection was limited. Samples were collected on three different days during a 2-wk period at the end of April and the first of May. During this period the University herd was on barn feeding.

RESULTS

The differences in milk composition between evening and morning samples for 645 cows in 11 herds are given in Table 1. These data show

TABLE 1

Average of the absolute differences between morning and evening percentages of milk fat, solids-not-fat, protein, and milk weights for 645 cows[a] in 11 herds

	Protein		Solids-not-fat		Milk fat		Milk weights	
	(%)		(%)		(%)		(lb)	
	[b]	[c]	[b]	[c]	[b]	[c]	[b]	[c]
All cows	−.03	.11	−.11	.26	− .73	.97	+4.21	4.32
12- to 12-hr intervals	−.03	.11	−.07	.25	− .31	.88	+1.06	2.26
10- to 14-hr intervals	−.03	.12	−.14	.29	−1.12	.89	+7.14	3.68
Holsteins	−.06	.11	−.17	.25	− .61	.68	+5.83	4.62
Guernseys	−.03	.12	−.14	.26	− .74	1.08	+3.60	3.94
Jerseys	−.00	.11	−.02	.25	− .84	1.09	+3.19	3.89
Winter	−.05	.12	−.17	.25	− .66	.83	+4.39	4.37
Summer	−.02	.10	−.06	.26	− .77	1.09	+4.03	4.27

[a] Some cows were included twice, since all cows in milk were sampled in winter and summer. A total of 1,290 individual milkings were sampled.

[b] Evening subtracted from morning (plus figures are higher for morning and minus figures higher for evening).

[c] Standard deviation of the differences between night and morning tests.

[d] Ten hours between morning and evening milkings—14 hr night and morning.

a tendency for milk weights to be higher and the percentage of milk constituents to be lower at the morning milkings. Milk fat percentages show much greater differences between evening and morning milk than either solids-not-fat or protein and more variation among individual cows as indicated by larger standard deviations.

Protein comparisons for the effects studied showed very little variation. In fact, there was no difference between milking intervals and only 0.03 percentage point difference betwee winter and summer months. Solids-not-f varied less in June than in January, but agai the differences were small.

Statistical analyses were made of the mil composition data of the 313 cows tested i the winter months by analysis of variance an Duncan's Multiple Range Test. The analys of variance was made by the method of fittin constants in a least-squares analysis as d

TABLE 2

Analysis of variance of evening and morning milk samples from 313 cows

Source	df	Mean squares Protein	 Solids-not-fat	 Milk fat	 Milk weights
		(%)	(%)	(%)	(lb)
Breed (B)	2	10.8135*	30.9136**	138.20**	2,561.07*
Interval (I)	1	1.1589	3.5187*	11.79	256.42
BI	2	.1102	.1825	1.48	231.50
Herds H:BI	8	1.5522**	.6434*	2.58**	404.83**
Cows C:HBI	299	.2567	.2714	.88	64.90
Time of day (T)	1	.4822**	4.5364**	71.06**	2,867.17**
BT	2	.1005	.8208	.19	131.29
IT	1	.0182	2.5709*	29.05**	1,565.83**
BIT	2	.0827	.1598	.91	12.00
TH:BI	8	.0397**	.2862**	2.17**	34.82**
TC:HBI	299	.0115	.0243	.20	2.74

* Significant at the 5% level.
** Significant at the 1% level.

TABLE 3

Means for evening and morning milkings from 313 cows

Protein (%)	Breed Means	Holstein 3.33	[a]	Guernsey 3.60		Jersey 3.79
	Time of day Means		Evening 3.60		Morning 3.55	
	Milking intervals Means			12-12 3.53		10-14 3.62
Solids-not-fat (%)	Breed Means	Holstein 8.63		Guernsey 9.22		Jersey 9.35
	Time of day Means		Evening 9.15		Morning 8.98	
	Milking intervals Means			12-12 9.00		10-14 9.13
Milk fat (%)	Breed Means	Holstein 4.01		Guernsey 5.35		Jersey 5.48
	Time of day Means		Evening 5.28		Morning 4.60	
	Milking intervals Means			12-12 4.82		10-14 5.06
Milk weights (lb)	Breed Means	Holstein 19.93		Guernsey 14.07		Jersey 13.91
	Time of day Means		Evening 13.79		Morning 18.18	
	Milking intervals Means			12-12 16.57		10-14 15.43

[a] Statistical significance at the 5% level tested by Duncan's Multiple Range Test. Any two means not underscored by the same line are significantly different. Any two means underscored by the same line are not significantly different.

scribed by Harvey (7). Due to unequal numbers, the tests are approximate. Results of these statistical analyses are given in Tables 2 and 3 and are discussed in the following paragraphs.

Protein. Herds, time of day, and herds by time of day were highly significant sources of variation for protein with breeds significant. Means of protein content of the milk were significantly different between Holsteins and Jerseys, but Guernseys were not significantly different from either of the two other breeds at the 5% level. Means of evening and morning samples were significantly different, but the means of the milking intervals were not significantly different for protein.

SNF. Breeds, time of day, and herds by time of day were highly significant sources of variation for solids-not-fat, while milking intervals and herds were significant. Solids-not-fat means were significantly different between Holsteins and the two other breeds but not between Guernseys and Jerseys. Means of evening and morning samples were significantly different, but means of milking intervals were not significantly different for solids-not-fat.

Fat. Breeds, herds, time of day, interval by time of day, and herds by time of day were all highly significant sources of variation for milk fat. Milk fat means were significantly different between Holsteins and the two other breeds, but not between Guernseys and Jerseys. Means of evening and morning samples were significantly different, but means of milking intervals were not significantly different for milk fat.

Milk weights. Herds, time of day, interval by time of day, and herds by time of day were highly significant sources of milk weight variation, whereas the differences between breeds were significant. Milk weight means were significantly different between Holsteins and the two other breeds, but not between Guernseys and Jerseys. Means of evening and morning milk weights were significantly different, as were the means of milking intervals.

Variation during milking process. Table 4 gives the means for the various groups on the four stages of milking and Tables 5 and 6 the statistical analysis of the data. A covariance analysis was necessary on the protein data to

TABLE 4

Means for stage of milking and time of day by breeds for milk components (whols milk basis)

Period *	Protein		Solids-not-fat		Fat		Milk weights	
	(%)		(%)		(%)		(lb)	
	PM	AM	PM	AM	PM	AM	PM	AM
			Ayrshires					
1	3.50	3.45	8.86	8.76	3.4	2.4	2.4	3.3
2	3.49	3.40	8.98	8.85	4.0	3.4	3.4	4.0
3	3.44	3.36	8.90	8.78	4.7	4.2	3.3	3.5
4	3.37	3.32	8.70	8.59	5.8	5.2	6.7	7.8
			Guernseys					
1	3.65	3.58	9.20	9.06	2.9	2.3	2.6	4.1
2	3.63	3.55	9.30	9.17	4.3	3.8	3.9	4.4
3	3.58	3.49	9.25	9.07	5.8	5.1	3.3	3.7
4	3.50	3.41	9.16	9.00	7.1	6.6	5.2	5.3
			Holsteins					
1	3.20	3.10	8.68	8.51	2.3	1.6	5.1	4.6
2	3.21	3.06	8.87	8.68	3.1	2.2	6.7	6.0
3	3.17	3.02	8.81	8.58	4.3	3.3	7.2	6.8
4	3.11	2.98	8.70	8.52	5.4	4.7	10.4	15.0
			Jerseys					
1	3.76	3.69	9.49	9.24	2.4	2.3	4.3	3.7
2	3.68	3.62	9.45	9.29	4.3	3.8	4.9	4.7
3	3.60	3.53	9.28	9.18	6.3	5.4	4.3	4.0
4	3.50	3.50	9.37	9.10	9.0	6.5	5.1	6.2
			Combined breeds					
1	3.53	3.45	9.06	8.89	2.8	2.2	3.6	3.9
2	3.50	3.41	9.15	9.00	3.9	3.3	4.7	4.8
3	3.45	3.35	9.06	8.90	5.3	4.5	4.5	4.5
4	3.37	3.30	8.98	8.80	6.8	5.8	6.9	8.6
Average per period	3.46	3.38	9.06	8.90	4.7	3.9	4.9	5.4

* Period 1 is the first minute of milking; Period 2 is the second minute of milking; Period 3 is the third minute of milking; Period 4 is the milk produced after 3 min of milking.

TABLE 5

Covariance analysis of stage of milking data for per cent protein

Per cent protein adjusted for milk weight			
Source	df		Mean square
Breed (B)	3		1.9322**
Time of day (T)	1		.3588**
Period (P)	3		.0800
Linear		1	1.0816**
Quadratic		1	.0160
Cubic		1	.0016
BT	3		.0145
BP	9		.0660
TP	3		.0274
BTP	9		.0120
Regression	1		1.8068**
Error	287		.0486

adjust for a significant decrease in protein percentage with increased milk weights.

The differences for time of day and breeds were statistically highly significant for protein and solids-not-fat as well as for milk fat. Stage of milking process was not significant for per cent protein, but was highly significant for solids-not-fat and milk fat, although the differences for solids-not-fat were relatively small. The largest differences found at the various stages of the milking process were 0.33% for protein, 0.81% for solids-not-fat, and 9.2% for milk fat.

There was a tendency for the percentage of protein to be slightly lower as milking progressed. Solids-not-fat increased from the first to the second period, but declined from the second to the fourth period. These solids-not-fat differences were statistically highly significant over-all as well as linearly and quadratically. The percentages of milk fat increased from the first to the fourth period. All three constituents showed statistically high significant differences between evening a morning milkings and were all lower in t morning.

TABLE 6

Analysis of variance of stage of milking data for per cent solids-not-fat and milk fat

			Mean square	
Source	df		Solids not-fat	Fat
			(%)	(%)
Breed (B)	3		6.9186**	42.65**
Time of day (T)	1		2.1946**	46.97**
Period (P)	3		.4322**	219.44**
Linear		1	.4489**	657.15**
Quadratic		1	.6994**	1.15
Cubic		1	.1482	.0004
BT	3		.0293	.73
BP	9		.0536	6.03**
TP	3		.0027	.83
BTP	9		.0118	1.71
Error	288		.0717	1.08

DISCUSSION

The variations generally found in this stu are similar to others reported in the literatu It appears that solids-not-fat variation is mu smaller than milk fat and protein is small than solids-not-fat. This is true in the compa sons of evening and morning milk as well in the different stages of the milking proce

The large increases in percentages of mi fat found as milking progresses agree with t work of Espe and Smith (4), Fosgate (5 Gardner et al. (6), Borsuk and Zaks (2), a Johansson et al. (8), although amounts m vary because of methods and individual c studies.

The higher evening milk fat percenta found in this study agree with the results Anderson (1), Fosgate (5), Nicholson et (11), and Thompson et al. (14), but do agree with those found by Specht et al. (1 and Lolkema and Have (10). As has been p viously pointed out, Specht worked with J seys and the milking hours were nearly ev In this study, herd differences were signific sources of variation and even milking hou showed less differences between evening a morning milk fat percentages than wide int vals of milking. These results are in agr ment with those of Schmidt and Trimberg (12). It is, therefore, possible that an in vidual herd milked at equal intervals co show no difference between evening and mor ing milk fat percentages.

The significant differences found betw evening and morning milk for solids-not- does not agree with the reports of Specht et (13), Nicholson et al. (11), and Thomp et al. (14). Since the differences in this stu were small, and the other reports were ba on results from relatively few cows and o one herd in each study, it is not surprising no significant differences were found by other workers.

The highly significant solids-not-fat perc age changes during the milking process do agree with the findings of Czako (3), Fos (5), or Gardner et al. (6), but again the dif ences were small. Differences in methods collecting samples could account for the dif ences found for solids-not-fat. Numbers methods were not given for Czako's (3) re Fosgate (5) took samples at the end of 30, and 120 sec of milking. Gardner et al. (6) fore milk, total milk between fore milk, the strippings for his samples.

Based on the results of this study, the differences in protein percentage between evening and morning samples are so small that there appears to be no need to obtain aliquot samples of evening and morning milk under a protein-testing program. It would be necessary, however, to obtain aliquot samples for solids-not-fat testing, since the SNF test is based on the milk fat test and the variation in milk fat is so great between night and morning milkings, especially in unequal milking intervals. Since the early stages of milking gave slightly higher tests for both protein and solids-not-fat, a representative milk sample should be taken from the entire milking under a testing program for either protein or solids-not-fat as well as for milk fat.

ACKNOWLEDGMENT

The authors gratefully acknowledge the fine cooperation of the 11 herd owners who provided the milk for this study. Also, special acknowledgment is made to Dr. Richard Damon, as Experiment Station Biometrician, for his assistance on the statistical analysis.

REFERENCES

(1) ANDERSON, A. C. Studies in the Range of Variation of the Per Cent of Butter Fat in the Milk of Individual Cows. Michigan Agr. Expt. Sta., Bull. 71. 1914.

(2) BORSUK, V. N., AND SAKS, M. G. Distribution of Butterfat and Casein in Consecutive Portions of Cows' Milk at a Single Milking. Trud. Inst. Fiziol. I. P. Pavlova, 4: 81. 1955. (In Russian.) (Dairy Sci. Abstr., 24: 248. 1962.)

(3) CSAKO, J. Study of Some Factors Affecting the Protein and S.N.F. Contents of Milk. Allattenyesztes, 10: 203. 1961. (Dairy Sci. Abstr., 24: 46. 1962.)

(4) ESPE, D., AND SMITH, V. R. Secretion of Milk. 4th ed. The Iowa State College Press, Ames. 1952.

(5) FOSGATE, O. T. Certain Factors Affecting the Solids-not-fat and Total Solids in Milk. J. Dairy Sci., 44: 980. 1961.

(6) GARDNER, R. H., THOMAS, W. R., AND WILLARD, H. S. A Study of Solids-not-fat Variations in Different Portions of Milk Drawn. Proc. 37th Annual Meeting Western Division, Am. Dairy Sci. Assoc. 1956.

(7) HARVEY, W. R. Least Squares Analysis of Data with Unequal Subclass Numbers. USDA, Bull. ARS-80-8. 1960.

(8) JOHANSSON, I., KORKMAN, N., AND NELSON, N. J. Studies on Udder Evacuation in Dairy Cows. I. The Rise in Fat Percentage During Milking. Acta Agr. Scand., 2: 43. 1952.

(9) KLIESCH, J., NEUHAUS, U., AND HORST, P. Investigations of the Variations in Protein and Butterfat Content of Milk in the Course of Lactation, and Relationships Between the Content of Protein, Butterfat and Milk Yield. Züchtungskunde, 31: 18. 1959.

(10) LOLKEMA, H., AND HAVE, A. J. VAN DER. The Determination of the Protein Content of Milk by Kofranyi's Method. Netherlands Milk Dairy J., 14: 277. 1960.

(11) NICHOLSON, W. S., JR., WILLARD, H. S., THOMAS, W. R., AND BROWN, D. C. Effect of Milking Time on Solids in Milk from Holsteins. J. Dairy Sci., 40: 1480. 1957.

(12) SCHMIDT, G. H., AND TRIMBERGER, G. W. Effect of Unequal Milking Intervals on Lactation Milk, Milk Fat, and Total Solids Production of Cows. J. Dairy Sci., 46: 19. 1963.

(13) SPECHT, L. W., BRUNNER, J. R., MADDEN, D. E., AND RALSTON, N. P. The Influence of Several Factors on the Protein and Solids-not-fat Content in the Milk of Jersey Cows. J. Dairy Sci., 39: 1337. 1956.

(14) THOMPSON, N. R., STONE, W. K., GRAF, G. C., KRAMER, C. Y., AND FREUND, R. J. Errors in Estimation of Lactational Yields of Milk, Fat, and Solids-not-fat from Individual Cows. J. Dairy Sci., 43: 951. 1950.

EFFECT OF DIETARY AND INJECTED FAT ON THE FATTY ACID COMPOSITION OF BOVINE DEPOT FAT AND MILK FAT[1, 2, 3]

S. B. TOVE AND R. D. MOCHRIE

Nutrition and Animal and Dairy Husbandry Sections, Department of Animal Science
North Carolina State College, Raleigh

SUMMARY

The ingestion of soybean oil by cows resulted in a distinct increase in stearic acid of both milk fat and adipose tissue, but oleic acid increased only in the milk fat. Linoleic acid increased only slightly when soybeans were fed, whereas intravenous infusion of a cottonseed oil emulsion produced a dramatic increase in the linoleate content of milk fat. These findings support the view that dietary unsaturated fatty acids are hydrogenated by the rumen microflora, then deposited by the animal. If the rumen is bypassed, unsaturated fatty acids are deposited without hydrogenation.

The inclusion of fat in the diet of ruminants, unlike animals with a simple stomach, has little effect upon the fatty acid composition of the depot fat of these animals. The relative constancy of fatty acid composition of ruminant depot fat presumably results from the action of rumen microflora in hydrogenating the unsaturated fatty acids present in the diet (3, 5, 8, 10). On the other hand, the increase in the iodine number of milk fat upon the addition of unsaturated fats to the diet is well established (1). Hilditch and Jasperson (4) showed that increases in the levels of stearic and oleic acids in milk fat resulted when fats high in linoleic acid were fed to cows. Observations of increased unsaturation of milk fat resulting from dietary fat, however, are inconsistent with an effective hydrogenation process in the rumen.

The feeding experiments reported herein were conducted to study changes in milk and depot fat simultaneously and to determine whether lactation would influence the deposition of unsaturated fatty acids. Parenteral administration of unsaturated fat, to bypass rumen action, was employed to compare the alteration in the fatty acid composition of milk fat with that from oral administration.

EXPERIMENTAL PROCEDURES

Soybean-feeding experiment. Four Holste cows were used in the study. Two of these an mals were lactating at the start of the exper ment and ceased to lactate near the midpoin Two others were not lactating at the start the experiment but came into lactation abo halfway through.

The animals were fed alfalfa hay and co silage ad libitum through the trial, which co sisted of a 2-wk preliminary period, a 9-w soybean-feeding period, and a 3-wk post-e perimental period. A standard concentra mixture consisting of 39% corn, 29% oa 20% soybean oil meal, and 10% citrus pu supplemented with sodium chloride and calciu and phosphorus, was fed in the prelimina period. Ground soybeans replaced 40% of t concentrate mixture during the first 3 wk the experimental period, after which the so bean content was raised to 60% for the remai ing 6 wk of the period. The average da intakes of soybeans were 2.4 lb for dry cows a 3.7 lb for lactating cows during the first 3 w and 4.7 and 6.8 lb in the last 6 wk. The fa acid compositions of the standard and of t soybean concentrate mixtures are given Table 1.

Samples of adipose tissue were taken fr the brisket by biopsy at weekly intervals. the same day, samples of the morning evening milk were pooled for fat extracti

Infusion experiment. Three trials were ducted in which a cottonseed oil emulsi was infused into the jugular vein of lacta

Received for publication February 23, 1963.

---

[1] Published with the approval of the Director of Research as Paper No. 1483 of the Journal Series.

[2] This investigation was supported in part by Grant A-2483 from the United States Public Health Service.

[3] A portion of this material was presented as part of the Milk Lipids Symposium at the 55th Meeting of the American Dairy Science Association at Logan, Utah. 1960.

[4] Lipomul I.V., provided by The Upjohn Kalamazoo, Michigan.

TABLE 1

Fatty acid composition of concentrate diets ingested and emulsion injected

Substance	Fat	16:0	18:0	18:1	18:2	18:3
		Fatty acids [a]				
	(%)					
Standard concentrate	4.1	18.8	4.0	38.2	39.0	....
Soybean concentrate	9.2	13.4	8.3	31.3	45.4	2.6
Lipomul	15.0	21.1	2.8	17.9	56.1	....

[a] Only fatty acids present at a level greater than 1.0% reported.

cows. The infusion was initiated immediately after the morning milking and was continued over a 2-hr period. In the first two trials, 450 g of cottonseed oil were infused, whereas 900 g were given in the third. A sample from each milking was collected for two days before and three or four days after the infusion. In the 450-g trials the cows were milked normally. In the trial when 900 g were given, the cows were first milked normally, then intravenously given 10 IU of oxytocin and milked again, to evacuate the glands more completely. For each milking the normal milk and milk obtained by oxytocin were pooled prior to sampling for fat extraction.

Analytical procedures. Milk fat was extracted by a mixture of ether and Skellysolve F after the addition of ethanol and ammonia (6). The adipose tissue samples were homogenized in ten volumes of a mixture of alcohol and ether (3:1), the solution brought to a boil, and the protein removed by filtration. The solvents from both the milk fat and depot fat extractions were removed on a steam bath, and the methyl esters of the fatty acids were prepared by transesterification (11). The fatty acids were analyzed by gas chromatography on a 4-ft column of Celite containing 30% of succinate–ethylene glycol polyester (12).

RESULTS

Soybean-feeding experiment. The inclusion of ground soybeans in the diet of Holstein cows altered the fatty acid composition of both the milk fat and depot fat (Figure 1). With milk fat, there were pronounced increases in the levels of stearic and oleic acids largely compensated for by decreases in the levels of myristic and palmitic acids. Although the percentage of linoleic acid in the milk fat increased slightly during the period of soybean feeding, this change was accompanied by a relatively large variation. The level of palmitoleic acid remained essentially constant irrespective of whether ground soybeans were present or absent in the diet. These results are in accord with the observation of Hilditch and Jasperson (4), that the ingestion of unsaturated fat by lactating cows results in an increase in the levels of stearic and oleic acids in milk fat. It is noteworthy that the level of linoleic acid in milk fat was only slightly increased, even though the dietary fat was richer in linoleic than it was in oleic acid. Moreover, the increase in the level of oleic acid can entirely account for the increase in iodine number observed by other investigators (1) with cows ingesting unsaturated fat.

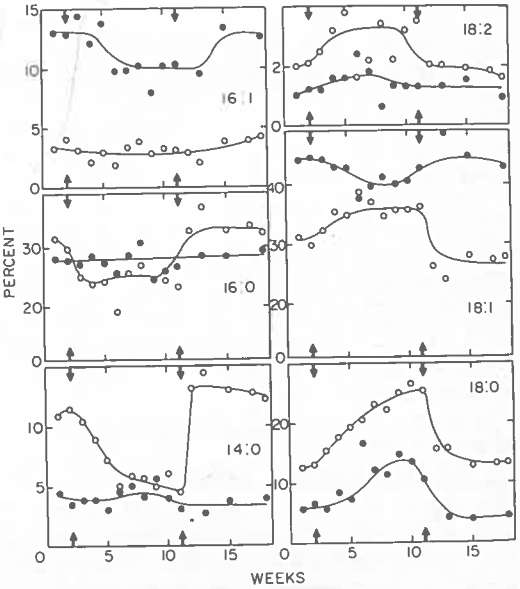


FIG. 1. Effect of soybean oil ingestion on the fatty acid composition of milk fat and adipose tissue. (The observations between the two arrows are for the period of soybean feeding.) ● adipose tissue (average of values for all four cows); ○ milk fat (average of values for the two cows lactating except in Weeks 7 and 8, when three cows were lactating).

The feeding of soybeans resulted in an increase in the level of stearic acid in brisket fat similar to that observed in milk fat, confirming the observations of Edwards et al. (2), who fed fat to steers. The level of linoleic acid in the depot fat was altered only slightly, if at all. Although the stearic acid and linoleic acid changes in the depot fat paralleled those observed in the milk fat, the depot fat levels of myristic, palmitic, palmitoleic, and oleic acids behaved quite differently from those of the milk fat. The level of both palmitic acid and myristic acid in the depot fat remained essentially constant throughout the course of the experiment. There was a marked decrease in the level of palmitoleic acid and a lesser decrease in the level of oleic acid in the depot fat associated with the soybean feeding.

The physiological process of lactation did not appear to influence the fatty acid composition of depot fat, since no changes in fatty acid composition coincident with the stage of lactation were observed.

Results of the soybean-feeding experiments, particularly the slight linoleic acid incorporation, were compatible with the idea that unsaturated fat was hydrogenated by the rumen microflora. However, the increase in oleic acid level in milk fat reported previously (4) and confirmed herein seemed to be in contradiction to this notion.

Infusion experiment. The intravenous infusion of 450 g or of 900 g, respectively, of a cottonseed oil emulsion dramatically raised the milk fat level of linoleic acid to a maximum of 12.5 and 22.0% (Figure 2). Although the fat infusion resulted in a decreased milk production for the collection immediately after the infusion, in each of the three studies the milk production returned to normal by 24 hr after the infusion, and there was no significant effect of the infusion on the milk fat percentage. Linoleic acid was the only acid to show an appreciable change upon fat infusion. This is illustrated by the data of Table 2, in whi the fatty acid composition of the milk befo infusion is compared to the average fatty ac composition of the two collection periods imm diately after infusion. In all three experimen the linoleic acid level in milk fat reached peak within one day following the infusi The maximum level reached when 900 g w administered was nearly double that of th 450-g infusion. Moreover, the linoleate le returned to normal after one day with th

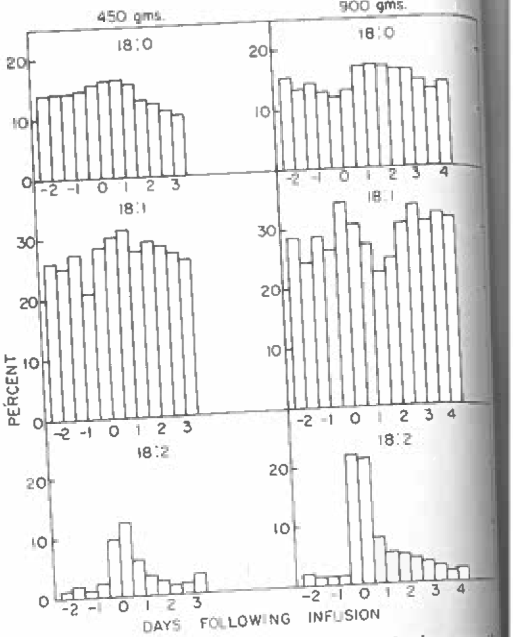


FIG. 2. Effect of cottonseed oil infusion on th levels of stearate, oleate, and linoleate in mi fat. Data given are averages of two cows th received 450 g cottonseed oil and one that r ceived 900 g.

TABLE 2

Fatty acid composition of milk fat immediately before and after cottonseed oil infusion

Fatty acid	Fat infused			
	450 g		900 g	
	Before infusion [a]	After infusion [b]	Before infusion [a]	After infusion [b]
	(%)			
14:0	13.5	10.2	13.2	8.2
14:1	2.2	0.8	2.4	0.9
16:0	39.7	33.8	37.8	30.6
16:1	2.6	1.5	2.4	1.8
18:0	11.4	10.1	13.2	9.5
18:1	26.3	28.0	25.5	24.5
18:2	2.6	13.9	1.8	21.6

[a] Average of four milkings.
[b] Average of two milkings.

450-g infusion, whereas with 900 g an elevated level of linoleate was maintained for several days, despite the use of oxytocin at each milking.

It is especially noteworthy that there was no change in the level of stearic acid (Figure 2) coincident with cottonseed oil infusion, and there was only a slight change in the level of oleic acid, despite the fact that the fat administered contained approximately 18% of oleic acid (Table 1).

DISCUSSION

The observation that feeding high levels of linoleic acid as supplied in the form of soybeans to cows results in little deposition of linoleic acid in either milk fat or adipose tissue, but that stearic acid is dramatically increased in both, is indicative of the efficiency and completeness of hydrogenation by the rumen microflora. Because the fatty acid composition of the tallow obtained from a steer raised on a low-fat diet was typical of that of normal beef tallow, Reiser et al. (9) brought up the possibility that the high stearate content of bovine depot fat might arise by hydrogenation of the C-18 unsaturated fatty acids by the animal. However, the observations following the infusion of cottonseed oil, where linoleic acid but not stearic acid was incorporated into milk fat, are indicative that hydrogenation of unsaturated fatty acids by the ruminant animal per se is not a significant biochemical process.

The difference in the effect of soybean oil ingestion on the fatty acid composition of milk fat and depot fat was particularly unexpected. The most striking difference was obtained with oleic acid which increased in milk fat with soybean feeding, whereas the level decreased in depot fat. Lauryssens et al. (7) have reported a significant conversion of stearic acid to oleic acid by a lactating mammary gland. It is possible that the increased oleic acid in the milk fat may have arisen by dehydrogenation of the stearic acid by the mammary tissue. If this is, indeed, the explanation, then it would follow that the dehydrogenation of saturated fatty acids to unsaturated fatty acids is considerably less in adipose tissue than in mammary tissue. The decreased level of palmitoleic acid and oleic acid in adipose tissue may reflect a specific replacement of these acids by stearic acid, and is in agreement with the results observed upon ingestion of stearic acid by mice.[5]

REFERENCES

(1) Deuel, H. J., Jr. The Lipids. Vol. II. p. 800. Interscience Publishers, Inc., New York. 1955.

(2) Edwards, R. L., Tove, S. B., Blumer, T. N., and Barrick, E. R. Effects of Added Dietary Fat on Fatty Acid Composition and Carcass Characteristics of Fattening Steers. J. Animal Sci., 20: 712. 1961.

(3) Garton, G. A. Lipid Metabolism in Herbivorous Animals. Nutrition Abstr. & Revs., 30: 1. 1960.

(4) Hilditch, T. P., and Jasperson, H. The Influence of Dietary Fat of Varying Unsaturation on the Component Acids of Cow Milk Fats. Biochem. J., 37: 238. 1943.

(5) Hoflund, S., Holmberg, J., and Sellmann, G. Inevstigation of Fat Digestion and Fat Metabolism in Ruminants. I. Feeding Unsaturated Fats to Dairy Cows; Fat Digestion in Rumen. Cornell Vet., 45: 254. 1955.

(6) Horwitz, W. Official Methods of Analysis. 9th ed. p. 190. Association of Official Agricultural Chemists, Washington, D. C. 1960.

(7) Lauryssens, M., Verbeke, R., and Peeters, G. Metabolism of Stearate-1-C^{14} in the Isolated Cow's Udder. J. Lipid Research, 2: 383. 1961.

(8) Reiser, R. Hydrogenation of Polyunsaturated Fatty Acids by the Ruminant. Federation Proc., 10: 236. 1951.

(9) Reiser, R., Choudhury, R. B. R., and Leighton, R. E. On the Origin of Stearic Acid in Ruminant Depot Fat. J. Am. Oil Chemists' Soc., 36: 129. 1959.

(10) Shorland, F. B., Weenink, R. O., Johns, A. T., and McDonald, I. R. C. The Effect of Sheep Rumen Content on Unsaturated Fatty Acids. Biochem. J., 67: 328. 1957.

(11) Tove, S. B., and Matrone, G. Effect of Purified Diets on the Fatty Acid Composition of Sheep Tallow. J. Nutrition, 76: 271. 1962.

(12) Tove, S. B., and Smith, F. H. Changes in the Fatty Acid Composition of the Depot Fat of Mice Induced by Feeding Oleate and Linoleate. J. Nutrition, 71: 264. 1960.

---

[5] S. B. Tove, unpublished observation.

METABOLISM OF UNIFORMLY LABELED GLUCOSE-C^{14} INTRODUCED INTO THE RUMEN OF A LACTATING COW. I. TRANSFER OF C^{14} TO RESPIRED AIR, VOLATILE FATTY ACIDS, AND MAJOR MILK CONSTITUENTS [1, 2]

K. K. OTAGAKI,[3] A. L. BLACK, J. C. BARTLEY,[4] M. KLEIBER, AND B. O. EGGUM [5]
School of Veterinary Medicine and The College of Agriculture
University of California, Davis

SUMMARY

To study the metabolic fate of dietary glucose in the lactating cow, uniformly C^{14}-labeled glucose (G-U-C^{14}) was introduced through a fistula into the rumen. Continuous samples of respired CO_2 were collected and assayed for carbon-14; in addition, samples of rumen and duodenal contents, jugular and ruminal vein blood, and milk were collected at frequent intervals and assayed for Carbon-14.

Following the administration of G-U-C^{14}, the specific activity reached a maximum in respired CO_2 at 45 min and in blood volatile fatty acids (VFA) at 15 min, but the maximum was not reached in plasma glucose until 2 hr had elapsed.

The first sample of rumen VFA, collected 1 hr after G-U-C^{14} administration, had the highest specific activity and later samples decreased in specific activity by approximately one-half each 4.5 hr. Carbon-14 was not detected in rumen VFA after 24 hr.

The specific activity of milk constituents after rumen administration of G-U-C^{14} was different from that observed after intravenous injection of G-U-C^{14}, but was similar to results obtained after intravenous injection of C^{14}-labeled short-chain fatty acids.

These results indicate that glucose was rapidly converted to short-chain fatty acids in the rumen before its absorption and utilization in the tissues of the cow for synthesis of milk components and oxidation to carbon dioxide.

This study presents new information on the difference in metabolic fate of dietary glucose compared to intravenously administered glucose in the ruminant and shows the rapid transfer of carbon from dietary glucose to blood VFA, which had not been previously demonstrated.

Considerable information relative to the m tabolism of carbohydrates in the ruminant h been reported in the literature. There is e dence that little, if any, glucose is assimila by ruminants through the gastrointestinal tr (5, 7, 15). This result has been attributed the rumen microflora which rapidly degra dietary carbohydrates to volatile fatty ac and gaseous products (1, 6, 11, 12). The V are absorbed into the bloodstream and, acco ing to quantitative estimates, provide a siderable proportion of the total energy quirement of the bovine (4, 14, 17).

Earlier studies with C^{14}-labeled glu (G-U-C^{14}) injected intravenously into the tating cow (2, 8) have shown that glucose pl an important role in the synthesis of milk stituents, principally lactose, but it prov

Received for publication January 14, 1963.

---

[1] This investigation was supported, in part, by a research grant RG-8183 from the Division of General Medical Science, P.H.S.

[2] Approved by the Director of the Hawaii Agricultural Experiment Station as Technical Paper No. 607.

[3] On leave from the University of Hawaii, Honolulu, during 1961-1962 academic year in the Department of Physiological Sciences, School of Veterinary Medicine. Held a Post-Doctoral Traineeship under PHS Training Grant 2G-646-PYTC from the Division of General Medical Sciences.

[4] Supported by PHS Post-Doctoral Fellowship, AF-11, 212 from the National Institute of Arthritis and Metabolic Diseases.

[5] Fellow of the Kellogg Foundation.

only a minor part of the cow's heat production. However, there is a paucity of information on the metabolic fate of C^{14}-glucose introduced directly into the rumen and the subsequent utilization of products formed in the rumen from glucose for milk synthesis. The latter study is of interest, because it reflects the influence of the rumen microflora on the utilization of dietary carbohydrate.

The present study was undertaken to obtain information on the transfer of C^{14} from G-U-C^{14} administered intraruminally to respired air, major milk constituents and VFA of blood, rumen, and duodenal contents. The data from respired air and major milk constituents are compared with those previously obtained when G-U-C^{14} was administered intravenously (8).

EXPERIMENTAL PROCEDURE

Cow. A 385-kg grade Holstein cow with a rumen fistula, duodenal enterostomy (20), and jugular and ruminal vein catheters was used. She was in her fourth month of lactation and produced 10 kg milk per day. The feeding regime consisted of alfalfa hay and a grain mixture of rolled barley, beet pulp, and protein supplements.

Tracer. Uniformly labeled glucose was synthesized by exposing *Canna indica* leaves to light in an atmosphere of $C^{14}O_2$ for a 24-hr period and then isolating the glucose according to the method of Putman and Hassid (16).

Tracer administration and dosage. A total of 12.45 mc of C^{14}-glucose (± 1 mg) dissolved in a liter of distilled water was distributed as evenly as possible throughout the rumen via the fistula. The total dose was added within 30 sec. The relative administered dose amounted to 32.34 μc C^{14}/kg body weight.

Unit for expressing standard units (s.u.) of specific radioactivity. The term s.u. is used here to designate the specific units expressed as microcuries per gram atom carbon in the various samples, divided by microcuries C^{14} injected per kilogram of body weight:

$$\text{s.u.} = \frac{\mu c\ C^{14}\ \text{per gram atom carbon}}{\mu c\ C^{14}\ \text{administered/kilogram body weight}}$$

This specific activity unit has the advantage that one may make direct comparisons between animals of different size that receive different amounts of carbon-14.

Analyses. A description of the respiration apparatus and the techniques for measuring CO_2 production and assay for carbon-14 in the respired CO_2 can be found in an earlier publication (9). Respired CO_2 was collected at 5- to 8-min intervals for 7 hr after the administration of G-U-C^{14} and intermittently thereafter for 40 hr. Rumen gases were collected by means of a plastic tube inserted through the rumen plug. A slight negative pressure in the rumen was maintained throughout the first 7 hr of the trial by use of Mariotte bottles. This arrangement was used to minimize the mixing by eructation of the respired CO_2 with gases produced in the rumen.

Milk was collected periodically during the first 35 hr after isotope administration and fractionated into its major organic constituents: casein, lactose, albumin, citric acid, and fat, and assayed for C^{14} as previously described (10).

The blood was centrifuged immediately after collection. The plasma was removed and deproteinized with $Ba(OH)_2$ and $ZnSO_4$ (19). The glucose concentration was determined by the glucose oxidase method (3). A portion of the concentrated protein-free filtrate was saved for steam distillation of VFA. Glucose was isolated from the remainder as glucosazone (2) and counted directly.

Rumen contents were collected from anterior, ventral and dorsal and the posterior, ventral and dorsal areas of the rumen at various times after adding G-U-C^{14}. Approximately 100 to 150 g of rumen contents were transferred directly into preweighed pint-sized Mason jars containing 150 ml of 1 N H_2SO_4. Volatile fatty acids were steam-distilled by a modification of the method described by McClendon (13) and fractionated on a silicic acid column, using chloroform and butanol-chloroform mixtures saturated with 0.05% HCl. The Packard Scintillation Spectrometer was used to assay C^{14} in VFA.

RESULTS

The specific activity of the cow's respired CO_2 changed with time, as shown by the curve in Figure 1. The maximum specific activity occurred about 40-50 min after the G-U-C^{14} was added to the rumen and decreased at an almost constant rate for several hours thereafter. In an earlier study (8), when G-U-C^{14} was injected intravenously into a lactating cow, the maximum specific activity occurred about 10 min earlier (see Figure 1) and was one and one-half times greater than that found after G-U-C^{14} was added to the rumen.

Figure 2 shows the change with time for the specific activity of plasma glucose collected from the ruminal and the jugular vein. During the first hour, the specific activities of glucose samples increased rapidly and the values were nearly the same in ruminal and jugular plasma.

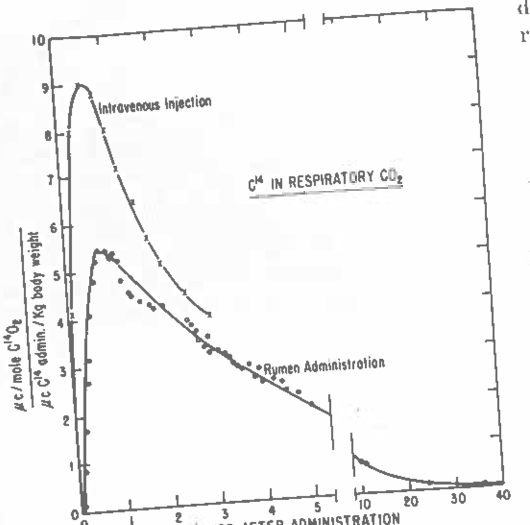


FIG. 1. Standardized activity of respired CO_2 after administration of G-U-C^{14}.

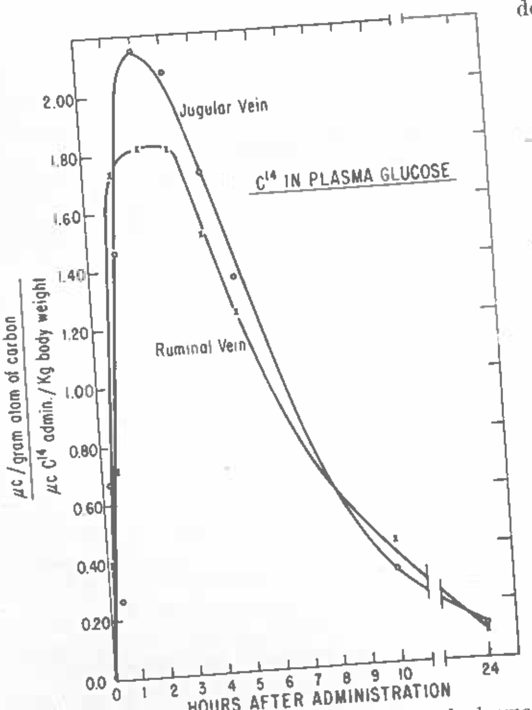


FIG. 2. Standardized specific activity of plasma glucose after administration of G-U-C^{14} into rumen.

In later samples, however, the glucose had a considerably higher specific activity in jugular blood and it was only after 10 hr had elapsed that the specific activity of glucose from jugular and ruminal vein again approached equality.

The specific activity of VFA recovered from different regions of the rumen at different periods of the trial are listed in Table 1. The

TABLE 1

Standardized specific activity of VFA from various parts of the rumen

Sample area	Time in hours			
	1	5	10	24
Posterior ventral	3.84	1.54	0.83	......
Anterior dorsal	2.71	1.49	0.22	0.03
Anterior ventral	3.01	1.20	0.95	0.13
Posterior dorsal	2.94	1.06	0.31	0.15

earliest samples collected, at 1 hr following addition of G-U-C^{14}, had the greatest specific activity. In later samples, the value decreased rapidly, indicating very fast turnover of the fatty acid pool.

Although the VFA recovered from rumen contents at the end of 1 hr had the greatest specific activity, it is apparent from the plasma determinations (Table 2) that the maximum

TABLE 2

Standardized specific activity of total plasma VFA at various time intervals

Blood sample	Time after adminis-tration	Jugular vein	Ruminal vein
	(hr)		
1	¼	2.17	6.26
2	½	0.30	3.73
3	1	0.95	3.82
4	2	1.21	3.59
5	3	0.39	2.97
6	4	0.15	1.30
7	5	0.21	1.25
8	12	0.03	0.10
9	24	0.01	......

specific activity in VFA had occurred m earlier. The VFA from jugular and rumi vein plasma had already reached maximum s cific activity in the first sample collected min after G-U-C^{14} was added to rumen conte Thus, it is apparent that the metabolism glucose in the rumen and absorption of f acids must be extremely rapid. The fact the specific activities of VFA obtained f the rumen and from ruminal vein blood w nearly equal at 1, 5, and 10 hr also ind rapid transfer of VFA from rumen to bl

The low specific activity of VFA isol from jugular plasma at one-half hour is i sistent with earlier and later samples fro jugular vein as well as samples from th minal vein. The specific activities of VFA from the ruminal vein were always greater than the VFA collected simultaneously from jugular plasma. One explanation for this difference would be that the liver selectively removed those fatty acids (18) containing most of the C^{14}.

The specific activities of VFA (Table 1) from samples collected in various parts of the rumen were not greatly different, indicating that the carbon-14 labeled glucose had been distributed rather uniformly throughout the rumen.

Separation of the rumen VFA into acetic, propionic, butyric and higher short-chain fatty acids showed that acetate had the highest specific activity. The ratio of specific activity of rumen VFA was approximately 7.4:1.3:1.3 for acetate, propionic, butyrate and higher VFA, respectively, for all collection periods. The specific activity of the individual VFA obtained from the posterior dorsal area was higher than those from the other areas of the rumen, with the exception of butyrate and higher VFA 1 hr post-administration of G-U-C^{14}. Similarly, the concentration of VFA in milliequivalents per 100 g of rumen contents was also highest in the sample obtained from the posterior dorsal area. Radioactivity, however, was greater in the posterior ventral region than in the other areas of the rumen throughout the collection periods, as shown in Table 1. One explanation for this difference might be attributed to the nonuniform distribution of the digesta, production of VFA therefrom, and unequal absorption rates from various areas of the rumen.

Samples collected from the duodenum between the third to fifth hour of the trial contained small amounts of carbon-14. Only an insignificant part of this C^{14} could be accounted for in VFA or glucose; most of the C^{14} was recovered in the fraction precipitated by addition of alcohol. This fraction was not identified chemically, but presumably contained mainly cellular material of a protein nature.

The transfer of carbon-14 to milk constituents is summarized in Figure 3. For purpose of comparison, data are included from earlier studies (8) on lactating cows injected intravenously with G-U-C^{14}. After adding G-U-C^{14} to the rumen, citric acid had the greatest specific activity, followed in decreasing order by milk fat, lactose, and casein. In contrast, when G-U-C^{14} was injected intravenously, lactose had the greatest specific activity and the value was about 40 times greater than the lactose recovered after adding G-U-C^{14} to the rumen. After intravenous injection of G-U-C^{14}, casein and citric acid also had greater specific activities and milk fat lower specific activities than the corresponding samples, after adding G-U-C^{14} to rumen contents.

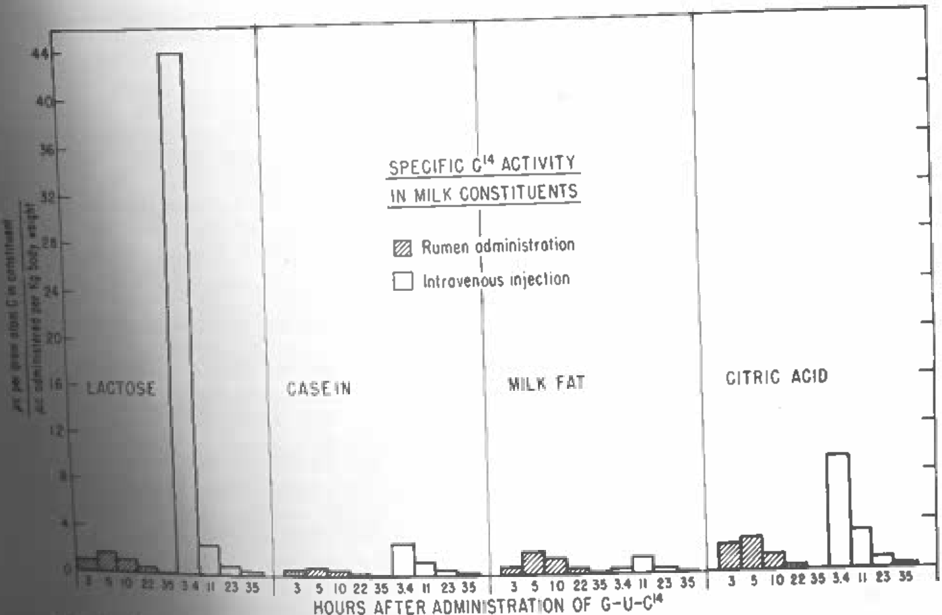


FIG. 3. Comparison of standardized specific activity of milk constituents after intravenous and intraruminal administration of G-U-C^{14}.

DISCUSSION

Results from this study clearly show that the metabolic fate of dietary glucose is distinctly different from that of blood glucose in the lactating cow. Most of this difference is presumably due to the metabolic activity of the rumen microflora, which readily attack glucose and convert the carbon to short-chain fatty acids and other products of fermentation.

It appears that little, if any, of the glucose per se which was added to the rumen was absorbed from the gastro-intestinal tract of the cow, even though carbon-14 appeared very early in respired CO_2. The maximum specific activity was not reached in the plasma glucose until 2 hr after the beginning of the trial. Meanwhile, the specific activity of respired CO_2 had reached a maximum at 45 min, and the short-chain fatty acids at 15 min, or perhaps even earlier.

The most reasonable explanation for these relationships would be that the G-U-C^{14} was fermented by the rumen microflora and the carbon of this glucose rapidly converted to short-chain fatty acids. These fatty acids were absorbed and, in part, oxidized in the cow's tissues, the C^{14} appearing in her respired CO_2. Additional fatty acid was apparently absorbed by the liver, as indicated by the lower specific activity of fatty acids from jugular plasma (post-liver) compared with fatty acids from simultaneous samples of ruminal vein plasma (pre-liver). Some of the fatty acid carbon, presumably mainly propionate, was converted to glucose and released from the liver into the blood. This conversion would account for the fact that the specific activity of glucose in jugular vein blood exceeded that of ruminal vein blood, at least during the interval from 2-8 hr after the G-U-C^{14} was added to rumen contents.

The best evidence that the G-U-C^{14} was extensively metabolized before its utilization in the cow's tissues comes from a comparison of the specific activity among the milk products synthesized. As shown by the histograms in Figure 3, the specific activities of all products were distinctly different after intravenous injection of G-U-C^{14} than they were after rumen addition of G-U-C^{14}. Intravenous G-U-C^{14} was transferred to lactose so efficiently that 47 to 55% of the total carbon-14 injected as G-U-C^{14} was recovered in this single product (8). After adding G-U-C^{14} to the rumen, only 2.6% of the C^{14} was recovered in lactose. This difference in results would not be expected if much of the C^{14} had been absorbed as glucose. On the other hand, milk fat contained, on the average, 7.5% of the C^{14} after intravenous injection of G-U-C^{14} (8), but accounted for 4.0% of the C^{14} recovered after rumen addition. When C^{14} acetate was injected intravenously into lactating cows, 9% of the C^{14} from acetate-1-C^{14} and 12% of the C^{14} from acetate-2-C^{14} was recovered in milk fat. Thus, the results after G-U-C^{14} was added to the rumen resemble more nearly the results after intravenous injection of C^{14}-acetate than results after intravenous injection of G-U-C^{14}.

The absence of appreciable amounts of C^{14} in the VFA or glucose fraction of duodenal contents is further evidence for extensive degradation of glucose in the rumen and absorption of the metabolic products from that organ. Most of the C^{14} in duodenal contents appeared to be in a protein fraction. Studies are currently under way to elucidate the utilization of glucose carbon for protein synthesis by rumen bacteria and protozoa.

ACKNOWLEDGMENTS

The authors acknowledge the valuable assistance of James A. Nakagiri, Edna Yeh, and Richard Gau. We are also indebted to Dr. Harold R. Parker for surgical services.

REFERENCES

(1) BARCROFT, J., MCANALLY, R. A., AND PHILLIPSON, A. T. Absorption of Volatile Fatty Acids from the Alimentary Tract of Sheep and Other Animals. J. Exptl. Biol., 20: 120. 1944.

(2) BAXTER, C. F., KLEIBER, M., AND BLACK, A. L. Glucose Metabolism in the Lactating Dairy Cow. Biochim. et Biophys. Acta, 17: 354. 1955.

(3) CAMPBELL, L. A., AND KRONFELD, D. S. Estimation of Low Concentrations of Plas Glucose Using Glucose Oxidase. Am. J. Vet. Research, 22: 587. 1961.

(4) CARROLL, E. J., AND HUNGATE, R. E. Th Magnitude of the Microbial Fermentation in the Bovine Rumen. Appl. Microbiol., 205. 1954.

(5) ELSDEN, S. R. The Fermentation of Carbohydrates in the Rumen of Sheep. J. Expt Biol., 22: 51. 1945.

(6) ELSDEN, S. R., AND PHILLIPSON, A. T. Ruminant Digestion. Ann. Rev. Biochem. 17: 705. 1948.

(7) HEALD, P. J., AND OXFORD, A. E. Fermentation of Soluble Sugars by Anaerobic Holotrich Ciliate Protozoa of the Genera I tricha and Dasytricha. Biochem. J., 506. 1953.

(8) KLEIBER, M., BLACK, A. L., BROWN, M. A., BAXTER, C. F., LUICK, J. R., AND STA MAN, F. H. Glucose as a Precursor of Mi Constituents in the Intact Dairy Cow. Bi chim. et Biophys. Acta, 17: 252. 1955.

(9) Kleiber, M., and Edick, M. A Respiration Apparatus for C^{14} Studies with Cows. J. Animal Sci., 11: 62. 1952.

(10) Kleiber, M., Smith, A. H., and Black, A. L. Carbonate as a Precursor of Milk Constituents in the Intact Dairy Cow. J. Biol. Chem., 195: 707. 1952.

(11) Lewis, D. Digestive Physiology and Nutrition of the Ruminant. Proc. Univ. Nottingham Seventh Easter School in Agr. Sci. 1960. Butterworth's, London. 1961.

(12) Lindsay, D. B. The Significance of Carbohydrates in Ruminant Metabolism. Vet. Rev. Annot., 5: 103. 1959.

(13) McClendon, J. F. Microdetermination of Volatile Fatty Acids in Blood. J. Biol. Chem., 154: 357. 1944.

(14) Pfander, W. H., and Phillipson, A. T. The Rates of Absorption of Acetic, Propionic and n-Butyric Acids. J. Physiol., 122: 102. 1953.

(15) Phillipson, A. T., and McAnnally, R. A. Studies on the Fate of Carbohydrate in the Rumen of Sheep. J. Exptl. Biol., 19: 199. 1942.

(16) Putman, E. W., and Hassid, W. Z. Isolation and Purification of Radioactive Sugars by Means of Paper Chromatography. J. Biol. Chem., 196: 749. 1952.

(17) Schambye, P. Experimental Estimation of the Portal Vein Blood Flow in Sheep. 2. Chronic Experiments in Cannulated Sheep Applying Infusion and Injection Methods. Nord. Vet. Med., 7: 1001. 1955.

(18) Shaw, J. C., Lakshmanan, S., Chung, A. C., Leffle, E. C., and Doetsch, R. N. Intermediary Metabolism in the Ruminant Including Studies on Rumen, Liver and Lactating Udder. Proc. 2nd Intern. Conf. Peaceful Uses of Atom. Energy, 27: 125. (Geneva.) 1958.

(19) Somogyi, M. Notes on Sugar Determinations. J. Biol. Chem., 195: 19. 1952.

(20) Wangensteen, O. H. Textbook of Surgery. p. 1945. Ed., F. Christopher. W. B. Saunders Co., New York. 1949.

NUTRITIVE VALUE OF THE DAIRY CATTLE RATION AS AFFECTED BY DIFFERENT LEVELS OF UREA AND QUALITY OF INGREDIENTS[1, 2]

N. F. COLOVOS, H. A. KEENER, H. A. DAVIS, B. S. REDDY,[3] AND P. P. REDDY[4]
Department of Dairy Science, New Hampshire Agricultural Experiment Station, Durham

SUMMARY

Two types of concentrate mixtures for dairy cattle, a low-fiber and a high-fiber, which contained four levels of urea, were fed together with two types of field-cured hay to three sets of unbred twin dairy heifers. The levels of urea were 0, 10, 20, and 40 lb per ton of concentrate mixture and the hays were harvested in the post-bloom stage the first year, and pre-bloom stage for the second and third years.

The nutritive evaluations of the 16 rations were made on the basis of digestible protein, total digestible nutrients, digestibile energy, metabolizable energy, and net energy, all directly determined.

Results of the experiment showed that without urea the low-fiber rations were generally superior to the high-fiber rations in digestible energy and total digestible nutrients, but when the level of urea in the concentrate mixtures was increased to 40 lb per ton, the high-fiber ration fed with early-cut hay was comparable to the low-fiber, more expensive concentrate mixture. The increase was due to a decrease in the heat increment as the urea in the ration was increased. The substitution of urea for protein did not affect the digestible crude protein content of the rations and had no significantly adverse effect on their nutritive values.

A 15% fiber concentrate mixture containing 40 lb of urea per ton fed as the sole ration also had a somewhat lower heat increment than a comparable ration containing no urea.

Urea was first suggested as a protein replacement for livestock feeds about 65 years ago and was used quite extensively in dairy feeds in Germany as early as World War I. It was theorized that microorganisms in the rumen act on the urea to convert it to protein. This theory was not generally accepted, however, until about 1937. Proof was based on both in vitro and in vivo studies. After this was clearly established much research was carried out to study the effect of different factors on the utilization of urea. Different species of ruminants were used in studies on growth and on milk and beef production, using urea as a partial protein replacement in the ration. Other work was carried out to study the toxicity of ure as well as the biological value of urea nitroge Reports in the literature (1, 2, 14, 15, 20), reviewed by Reid (24), justified the conclusion as early as 1953, that urea can safely and satisfactorily replace at least one-fourth of the pr tein nitrogen of the ration for dairy cattle.

During the last few years, urea has assumed an important role as a protein replacement in concentrate mixtures for ruminants, partic larly cattle, as a result of additional resear the results of which were published since th above review (3-7, 12, 15, 18, 20). Field ob servations, mostly by feed company dairy sp cialists and extension men, and also carefull controlled in vitro and in vivo experiment have indicated that the inclusion of urea certain types of rations resulted in benefic nutritional effects in addition to the prote value (7, 9, 10, 21-23, 28). However, there no general agreement as to type and qual of concentrate mixture or forage with wh urea-containing mixtures should be fed to g these beneficial results. Some feed manuf turers believe that urea can be utilized effectively in high-quality, low-fiber con

---

Received for publication October 28, 1962.

[1] Published with the approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 299.

[2] This research was partially supported by a grant from the E. I. DuPont deNemours and Company of Wilmington, Delaware.

[3] Present address: Dairy Department, Michigan State University, East Lansing.

[4] Present address: Dairy Husbandry Department, University of Maryland, College Park.

trate mixtures; whereas, others use it in economy-type high-fiber mixtures.

Even though a large amount of research has been carried out on other phases of urea utilization, little research has been conducted on the relationships between levels of urea used, quality of ingredients, and the effect on the digestibility and utilization of the protein and energy of the ration.

This study was undertaken, therefore, to determine the quality of ration in which urea is utilized most effectively and the optimum level of urea in rations of various qualities.

EXPERIMENTAL PROCEDURE

In the main studies two types of concentrate mixtures were used, a low-fiber mixture containing approximately 5% fiber and a high-fiber mixture containing approximately 10% fiber. To each type of concentrate mixture were added four levels of urea, 0, 10, 20, and 40 lb per ton, or the equivalent of 0, 8.75, 17.5, and 35% of the protein of the concentrate mixture. In addition, two 15% fiber concentrate mixtures, one containing no urea and the other 40 lb per ton, were fed as the sole rations in heat increment studies.

The formulae and chemical composition of the concentrate mixtures used for the nutritive evaluation experiments taken from Morrison's book are shown in Table 1. The variables used in standardizing the concentrate mixtures for protein and energy were urea, cornmeal, and soybean oil meal. Oat feed was substituted for the crimped oats to increase the fiber content. The amounts of all the other ingredients in the mixtures remained constant. All the ingredients for the concentrate mixtures were bought at one time, with assurances from the dealer that they were from the same lot, to prevent lot-to-lot variation in the different grains. A batch, sufficient to carry one animal through the digestion and utilization experiment, was mixed each time as needed. The actual analyses of the concentrate mixtures and forages are shown in Table 2.

The forages fed with the above concentrate mixtures were late-cut field-cured mixed grass hay during the first year, 1959-1960, and early-cut field-cured grass hay during the next two years, 1960-1961 and 1961-1962.

Three sets of unbred twin dairy heifers were used as experimental subjects. One set was identical Ayrshires and the other two were fraternal twin Holsteins. The first year, 1959-1960, the identical-twin Ayrshires, 22 months of age and about 900 lb, and one set of fraternal twin Holsteins, 18 months of age and weighing about 700 lb at the beginning of the experiment, were used to evaluate the rations. In the second year's experiments, 1960-1961, the same identical twin Ayrshires, weighing about 1,050 lb, and a different set of fraternal twin Holsteins, 20 months of age and weighing

TABLE 1

Concentrate mixtures (1959-1960, 1960-1961)

	Urea levels							
	High energy				High fiber			
	0	10	20	40	0	10	20	40
	(lb)							
Corn meal	825	880	940	1,030	735	795	855	975
Crimped oats	400	400	400	400	......	......	......	......
Oat feed	......	......	......	......	400	400	400	400
Wheat bran	200	200	200	200	200	200	200	200
Corn gluten feed	50	50	50	50	50	50	50	50
Brewers grain	50	50	50	50	50	50	50	50
Cane molasses	200	200	200	200	200	200	200	200
Soybean oil meal	245	180	110	......	335	265	295	55
Urea	0	10	20	40	0	10	20	40
Dicalcium phosphate	10	10	10	10	10	10	10	10
Trace mineralized salt	20	20	20	20	20	20	20	20
Total	2,000	2,000	2,000	2,000	2,000	2,000	2,000	2,000
	(%)							
Crude protein	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Digestible protein	12.2	12.4	12.4	13.1	12.5	12.6	12.7	12.7
T.D.N.	73.2	73.2	73.1	72.4	68.3	66.3	66.2	66.2
Fiber	5.4	5.3	5.2	5.0	9.2	9.1	8.9	8.7
Fat	3.3	3.4	3.5	3.6	2.5	2.6	2.6	2.8
Ca	.21	.20	.19	.18	.24	.23	.22	.20
P	.52	.50	.48	.47	.50	.49	.48	.45

TABLE 2

Proximate analysis and caloric content of feeds[a]

Feeds	Ash	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Gross energy
	(%)					(Cal/g)
1959-1960						
Mixed grass hay	6.10	6.30	2.26	34.25	51.02	4.4051
Low-fiber grain mixtures						
0 lb urea per ton	5.91	17.55	3.92	5.66	66.96	4.3726
10 lb urea per ton	5.66	17.25	3.90	5.59	67.59	4.3338
20 lb urea per ton	5.28	17.24	4.03	5.80	68.00	4.3964
40 lb urea per ton	5.57	17.16	4.27	5.21	67.95	4.3338
High-fiber grain mixtures						
0 lb urea per ton	6.46	16.99	2.76	10.60	63.20	4.3539
10 lb urea per ton	6.77	17.39	2.88	10.10	63.03	4.3487
20 lb urea per ton	6.40	17.14	2.97	10.06	63.42	4.3151
40 lb urea per ton	5.80	16.83	3.16	9.99	64.23	4.3054
1960-1961						
Early-cut hay	7.43	14.31	2.96	34.46	40.84	4.4287
Low-fiber grain mixtures						
0 lb urea per ton	5.91	18.31	3.62	5.18	66.96	4.3351
10 lb urea per ton	5.56	18.39	3.77	5.48	66.79	4.3326
20 lb urea per ton	5.55	18.17	3.84	5.16	67.29	4.3061
40 lb urea per ton	4.64	18.05	4.06	4.55	68.71	4.3515
High-fiber grain mixtures						
0 lb urea per ton	6.47	18.29	2.74	9.24	63.11	4.3259
10 lb urea per ton	6.47	18.40	2.93	10.17	62.04	4.3385
20 lb urea per ton	6.24	18.68	2.98	9.18	62.92	4.3137
40 lb urea per ton	5.97	17.66	3.20	9.75	63.21	4.2479

[a] Values expressed on dry matter basis.

about 950 lb, were the experimental animals. Every animal received every ration an equal number of times, a complete balanced design. Examination of the means within the twin pairs seemed to show no differences between twins or within twins on the same ration.

The procedures used to study the nutritive value of the experimental rations were those routinely followed in this laboratory (8, 25, 26). They consist mainly of the following:

1. *Preliminary period.* This is an adjustment period of 15 to 18 days during which the animal becomes accustomed to the kind and amount of feed to be evaluated. The level of nutrition at which the evaluation is carried out is slightly above maintenance.

2. *Collection period.* This period lasts eight to ten days. During this time the feed, feces, and urine are weighed, sampled, and a composite sample of each is kept for analysis. The feces are preserved by freezing to a solid state and the urine is kept under refrigeration, just above freezing.

3. *Heat production measurement on feed.* After the collection period, the animal is put in the respiration chamber, fed the same amount of feed as during the collection period, and its heat and methane production measured by means of indirect calorimetry. By means of an electric eye, the length of time the animal spends standing and lying is recorded. Fro this record it is determined whether the acti ity is normal. Heat production measurement are based on 24 hr of normal activity. minimum of 35% lying and a maximum about 12 changes of position are the limi for a normal period (12).

4. *Heat production on fast.* The heat produ tion of the animal is measured after it has be fasted for 48 hr. A post-absorptive conditi is considered to have been reached when me ane ceases to be produced and the respirator quotient (ratio of carbon dioxide produced oxygen consumed) is 0.75 or less. At the le of nutrition of this experiment, this is reach at 48 hr after last feed is consumed.

The difference between heat production feed and heat production on fast is the h increment of the ration or work of digestio

RESULTS AND DISCUSSION

The digestion and utilization experim were carried out between October and Jun 1959-1960 and 1960-1961. They were pla so that each one of the eight rations woul fed to four animals according to the sch shown in Table 4.

Results of the first two years' work, 19 1960 and 1960-1961, are shown in Tabl The 1959-1960 experiments, which cons

TABLE 3

Concentrate mixtures fed as sole ration in 1961-1962

	0 lb urea per ton	40 lb urea per ton
	(lb)	
Corn meal	300	550
Oat feed	750	800
Wheat bran	200	200
Corn gluten	100	50
Brewer's grain	100	50
Cane molasses	200	200
Soybean oil meal	320	80
Urea	0	40
Dicalcium phosphate	10	10
Trace mineralized salt	20	20
Total	2,000	2,000
	(%)	
Protein	14.6	14.6
Fiber	15.1	15.0
Fat	2.9	2.6

of 32 complete digestion and utilization balances and 64, 24-hr heat production measurements in the respiration chamber, showed the following:

The digestibility of the dry matter, total digestible nutrients, digestible energy, metabolizable energy, and net energy were higher in the low-fiber rations than they were in the comparable high-fiber rations. Digestibility of protein was about the same in comparable low- and high-fiber rations and was not affected by level of urea. As the level of urea in the ration was increased, the net energy of the high-fiber ration increased from 1.507 Calories per gram in the 0 urea to 1.625 Calories per gram in the 40 lb urea, per ton concentrate mixture.

In the second year's experiments, 1960-1961, when the same concentrate mixtures were fed with the early-cut hay, a statistical analysis of these results show a significant increase in net energy as the urea was increased from 0 to 40 lb per ton in the concentrate mixture. The 1960-1961 results further confirm those of the previous year, in that the low-fiber rations were superior in total digestible nutrients, digestible energy, metabolizable energy, and net energy, and that addition of urea in the concentrate mixture up to 40 lb per ton did not have any observed adverse effect in any of the rations.

The most noteworthy observation the second year was that when urea was increased to 40 lb per ton in the high-fiber mixture it was equal to any of the low-fiber mixtures in net energy value. The net energy of the high-fiber ration without urea contained 1.489 Calories per gram, but increased to 1.740 Calories per gram in the ration containing 40 lb urea per ton.

The forage used the first year was of poor-quality late-cut mixed grass hay, whereas in the second year the forage was early-cut mixed grass hay with a higher protein content. In both years the concentrate mixtures were about the same. This suggests, then, that the addition of urea will not provide significant improvement to poor forages coupled with high-fiber concentrate mixtures, but will improve the net energy values of rations consisting of good-quality forage and the same concentrate mixtures. The greatest change in net energy values seemed to be at the lower levels of urea, and the addition of urea in amounts in excess of 20 lb per ton lead to smaller incremental

TABLE 4

Schedule of experiments of urea study

Animals	Series 1 Oct.-Nov.	Series 2 Nov.-Dec.	Series 3 Dec.-Jan.	Series 4 Jan.-Feb.	Series 5 Feb.-Mar.	Series 6 Mar.-Apr.	Series 7 Apr.-May	Series 8 May-June
				1959-1960 rations				
A 338	E1	E4	F3	F1	F4	E3	F2	E2
A 339	E3	F4	F1	F3	E4	E1	F2	E2
H 357	E2	F1	E1	E3	F3	F2	F4	E4
H 358	F4	E3	F2	E2	F1	F3	E1	E4
				1960-1961 rations				
A 338	E2	F2	E3	F4	F1	F3	E4	E1
A 339	F2	E2	F3	E1	F1	E4	F4	E3
H 434	F1	F2	E4	F4	F3	E1	E3	E2
H 435	F3	F1	E1	E2	E3	F4	F2	E4

E1 = Low-fiber 0 lb urea/ton concentrate mixture.
E2 = Low-fiber 10 lb urea/ton concentrate mixture.
E3 = Low-fiber 20 lb urea/ton concentrate mixture.
E4 = Low-fiber 40 lb urea/ton concentrate mixture.
F1 = High-fiber 0 lb urea/ton concentrate mixture.
F2 = High-fiber 10 lb urea/ton concentrate mixture.
F3 = High-fiber 20 lb urea/ton concentrate mixture.
F4 = High-fiber 40 lb urea/ton concentrate mixture.

TABLE 5

Average nutritive values of entire rations

Concentrate mixtures	TDN		Metabolizable energy	Net energy	Digestibility of crude protein	Digestibility of dry matter
	(%)	(Cal per g)			(%)	(%)
		Rations including late maturity hay (1959-1960)				
Low-fiber						
Urea, 0 lb/ton	69.37	3.017	2.509	1.739	68.85	69.27
Urea, 10 lb/ton	70.52	2.953	2.417	1.657	67.41	70.03
Urea, 20 lb/ton	69.25	2.951	2.500	1.742	65.90	68.32
Urea, 40 lb/ton	71.23	2.987	2.535	1.777	68.44	70.46
High-fiber						
Urea, 0 lb/ton	63.79	2.704	2.255	1.507[a]	69.47	64.10
Urea, 10 lb/ton	63.10	2.713	2.170	1.492	66.91	63.82
Urea, 20 lb/ton	63.63	2.707	2.211	1.533	64.39	63.64
Urea, 40 lb/ton	63.39	2.648	2.173	1.625	66.00	62.78
		Rations including early maturity hay (1960-1961)				
Low-fiber						
Urea, 0 lb/ton	75.90	3.072	2.433	1.713	73.80	77.01
Urea, 10 lb/ton	76.54	3.128	2,503	1.686	73.52	76.76
Urea, 20 lb/ton	75.42	3.053	2.425	1.712	74.37	76.90
Urea, 40 lb/ton	75.75	3.043	2.433	1.739	73.34	76.04
High-fiber						
Urea, 0 lb/ton	70.69	2.827	2.233	1.489	73.99	72.02
Urea, 10 lb/ton	71.92	2.896	2.252	1.606	74.05	73.77
Urea, 20 lb/ton	71.55	2.874	2.314	1.650	74.32	72.78
Urea, 40 lb/ton	70.93	2.856	2.352	1.740	72.92	72.31

[a] Average of three animals only.

changes in net energy values.

The results of the experiment, therefore, indicate that, when fed with early-cut grass hay and on the basis of net energy, the high-fiber ration with 40 lb urea per ton, which contained less expensive ingredients, was approximately comparable to the low-fiber concentrate mixtures which contained more expensive ingredients.

This confirms field observations that urea used in limited amounts in certain types of rations has beneficial nutritional properties in addition to the protein it supplies. It is also in agreement with results obtained at this Station on vitamin synthesis as affected by urea, which showed that the synthetic activity improved significantly at the higher urea levels, particularly in vitamin B_{12} (28).

To shed some light on the reason net energy increased in the higher fiber rations with the addition of urea, and whether the trend of lower heat increment persisted, 15% fiber concentrate mixtures with 0 and 40 lb urea per ton formulated as shown in Table 3 were similarly evaluated. The heat production of each animal was measured after about a month on each of these concentrate mixtures as the sole ration. From the heat production measurements on feed and on fast the heat increment values were calculated. These are shown in Table 6. The results show a considerable decrease in the heat increment when the animals were fed the concentrate mixture containing the urea.

To study further this effect of urea on the heat increment, 24 complete digestion and utilization balance experiments were conducted during the year 1961-1962. Results of these experiments, which are shown in Table 7, are summarized as follows:

Results from feeding both 5 and 10% fiber concentrate mixtures containing 0 and 40 lb urea per ton essentially confirmed the results of the previous year, in which the high-fiber rations containing this level of urea had about the same net energy per unit weight of feed as the low-fiber feed. Actually, the net energy of the low-fiber was 1.806 Calories per gram and the high-fiber 1.752 Calories per gram; however, an analysis using Student's "t" test showed no significance. The daily balance of energy was slightly higher with the low-fiber mixtures, but the reverse was true in the daily balances of the protein.

With the all-concentrate rations containing urea, the net energy and daily balance of energy were higher in the low-fiber ration. However, the digestibility and daily balance of protein were high in the high-fiber ration.

In all-concentrate rations containing no urea

TABLE 6

Heat increment of 15% fiber ration with and without urea 1961-1962

Ration	Animal	Heat production per 24 hours On feed	 On fast		Heat increment of ration
		————	(Cal)	————	
0 Urea/ton	A 338	14,247	9,648		4,599
	H 434	14,342	10,861		3,481
	H 435	12,073	9,288		2,785
				Avg	3,622
40 lb Urea/ton	A 338	12,104	8,762		3,342
	H 434	13,726	10,263		3,463
	H 435	11,658	9,252		2,406
				Avg	3,070

TABLE 7

Average nutritive values of urea rations 1961-1962

Ration	Dry matter digestibility	Digestible energy	Metabolizable energy	Net energy	Daily energy balance	Digestibility protein	Daily protein balance
	(%)	————	(Cal per g)	————	(Cal)	(%)	(g)
Low-fiber 40 lb urea/ton + hay	75.90	3.185	2.677	1.806	+ 3,469	71.36	+ 100
High-fiber 40 lb urea/ton + hay	70.06	2.897	2.423	1.752	+ 2,817	68.18	+ 113
Low-fiber 40 lb urea/ton as sole ration	84.20	3.580	3.110	2.359	+ 3,365	79.11	+ 165
High-fiber 40 lb urea/ton as sole ration	75.24	3.167	2.608	1.850	+ 731	84.18	+ 182
Low-fiber 0 lb urea/ton as sole ration	83.93	3.624	3.070	2.260	+ 3,981	77.60	+ 182
High-fiber 0 lb urea/ton as sole ration	73.07	3.041	2.523	1.803	+ 365	77.33	+ 135

the net energy and daily balance of the high energy mixture were likewise significantly higher than in the high-fiber mixture.

Results of this experiment indicate that the inclusion of urea in a concentrate mixture for dairy cattle fed with early-cut hay tends to increase the net energy value of the ration, apparently due to a decreased loss of energy in the heat increment. The substituting of urea for part of the protein has essentially no effect on the digestible protein content. These differences and their significance are shown in Table 8.

REFERENCES

(1) Archibald, J. G. Feeding Urea to Dairy Cows. Massachusetts Agr. Expt. Sta., Bull. 406. 1943.

(2) Bartlett, S., and Blaxter, K. L. The Value of Urea as a Substitute for Protein in the Rations of Dairy Cattle. J. Agr. Sci., 37: 32. 1947.

(3) Belasco, I. J. New Nitrogen Feed Compounds for Ruminants—A Laboratory Evaluation. J. Animal Sci., 13: 601. 1954.

✓(4) Belasco, I. J. Comparison of Urea and Protein Meals as Nitrogen Sources for Rumen Microorganisms: The Production of Volatile Fatty Acids. J. Animal Sci., 13: 748. 1954.

✓(5) Belasco, I. J. Comparison of Urea and Protein Meals as Nitrogen Sources for Rumen Microorganisms. Urea Utilization and Cellulose Digestion. J. Animal Sci., 13: 739. 1954.

✓(6) Belasco, I. J. The Role of Carbohydrates in Urea Utilization, Cellulose Digestion and Fatty Acid Formation. J. Animal Sci., 15: 496. 1956.

✓(7) Bell, M. C., Gallup, W. D., and Whitehair, C. H. Value of Urea Nitrogen in

TABLE 8

Average heat increments and daily balances of energy and protein

Concentrate mixtures	Rations including late-maturity hay (1959-1960)			Rations including early-maturity hay (1960-1961)		
	Heat increment	Energy balance	Protein balance	Heat increment	Energy balance	Protein balance
	(Cal)	*(Cal)*	*(g)*	*(Cal)*	*(Cal)*	*(g)*
Low-fiber						
Urea, 0 lb/ton	4,274	4,970	143	4,189	2,354	143
Urea, 10 lb/ton	4,478	4,798	127	4,686	2,281	119
Urea, 20 lb/ton	4,333	4,878	117	4,046	2,201	125
Urea, 40 lb/ton	4,532	4,784	115	4,067	2,120	145
High-fiber						
Urea, 0 lb/ton	4,572	2,064	107	4,305	792	111
Urea, 10 lb/ton	4,118	1,641	125	3,861	1,638	99
Urea, 20 lb/ton	4,148	1,827	125	3,864	1,721	128
Urea, 40 lb/ton	3,939	1,034	124	3,611	1,691	150

Rations Containing Different Carbohydrate Feeds. J. Animal Sci., 12: 787. 1953.

(8) Benedict, F. G., Coropachinsky, V., and Ritzman, E. G. Technik der Gesamtstoffwechsels und des Energiebedarfes von Haustieren. Abderhalden's Hand d. biolog. Arbeitmedhoden, Abt. IV, Teil 13: 619-687. 1934.

(9) Briggs, H. M., Gallup, W. M., Dining, J. S., Darlow, A. E., Stephens, D. F., and Campbell, W. D. The Efficiency of Urea as a Protein Extender. Oklahoma Agr. Expt. Sta., Misc. Publ., 15: 1125. 1956.

(10) Campling, R. C., Freer, M., and Balch, C. C. The Effect of Urea on the Voluntary Intake of Oat Straw. Brit. J. Nutrition, 15: 531. 1961.

(11) Campling, R. C., Freer, M., and Balch, C. C. Factors Affecting the Voluntary Intake of Food by Cows. Brit. J. Nutrition, 16: 115. 1962.

(12) Fuller, J. M. Some Physical and Physiological Activities of Dairy Cows. New Hampshire Agr. Expt. Sta., Tech. Bull. 35. 1928.

(13) Green, D. F. Safety of Using Urea in Ruminant Feeds. Feedstuffs Magazine, January 15, 1955.

(14) Harris, L. E., and Mitchell, H. H. The Value of Urea in the Synthesis of Protein in the Paunch of the Ruminant. J. Nutrition, 22: 167. 1941.

(15) Hastings, W. H. The Use of Urea in Commercial Dairy Feeds. J. Dairy Sci., 27: 1015. 1944.

(16) Lassiter, C. A., Grimes, R. M., Duncan, C. W., and Huffman, C. F. High Level Urea Feeding to Dairy Cattle. J. Dairy Sci., 41: 281. 1958.

(17) Loosli, J. K. Urea is Safe and Useful Protein Feed. Hoard's Dairyman, March 10, 1962.

(18) Loosli, J. K., and Warner, R. G. Distillers Grains, Brewers Grains, and Urea as Protein Supplements for Dairy Rations. J. Dairy Sci., 41: 1446. 1958.

(19) Morrison, F. B. Feeds and Feeding. 22nd ed. Morrison Publ. Co., Ithaca, N. Y. 1956.

(20) Owen, E. G., Smith, J. A. B., and Wright, N. C. Urea as a Partial Protein Substitute in Feeding of Dairy Cattle. Biochem. J., 37: 44. 1943.

(21) Ratcliff, L., and Stallcup, O. T. Urea as a Protein Substitute in Feeds for Dairy Heifers. Proc. Assoc. Southern Agr. Workers, 51st Ann. Conv., p. 79. 1954.

(22) Reddy, B. S. The Effects of Different Levels of Urea on the Nutritive Value of a High Fiber Concentrate Mixture when Fed with Average Hay to Dairy Cattle. Master's thesis, University of New Hampshire. 1960.

(23) Reddy, P. P. The Effects of Different Levels of Urea on the Nutritive Value of a High Fiber Concentrate Mixture When Fed with Early Cut Hay to Dairy Cattle. Master's thesis, University of New Hampshire. 1962.

(24) Reid, J. T. Urea as a Protein Replacement for Ruminants. A Review. J. Dairy Sci., 36: 955. 1953.

(25) Ritzman, E. G., and Benedict, F. G. Nutritional Physiology of the Adult Ruminant. Carnegie Inst. Wash., Publ. 494. 1938.

(26) Ritzman, E. G., and Colovos, N. F. An Automatic Method for Collecting Solid and Liquid Excreta from Cows in Digestion Experiments. New Hampshire Agr. Expt. Sta., Tech. Bull. 52. 1932.

(27) Rupel, I. W., Bohstedt, G., and Ha
E. B. The Comparative Value of Urea an
Linseed Meal for Milk Production. J. Dai
Sci., 26: 647. 1943.

(28) Teeri, A. E., and Colovos, N. F. Eff
of Urea in the Ration of Cattle on Vitam
Synthesis in the Rumen. J. Animal S
(In press.) 1963.

(29) Tillman, A. D., and Swift, R. W.
Utilization of Ammoniated By-products
Urea by Sheep. J. Animal Sci., 12:
1953.

EFFECT OF DIETARY FAT AND MINERALS ON THE INCIDENCE OF DIARRHEA AND RATE OF PASSAGE OF DIETS IN THE DIGESTIVE TRACT OF DAIRY CALVES[1]

L. J. BUSH, J. D. SCHUH,[2] N. B. TENNILLE, AND G. R. WALLER
Department of Dairy, Oklahoma Agricultural Experiment Station, Stillwater

SUMMARY

Effects of adding minerals simulating whey ash, animal tallow, or both to a basal nonfat milk solids diet for calves were determined. Highly significant differences ($P < 0.01$) in the over-all incidence of diarrhea were observed among groups of calves fed the different diets in one trial extending for 21 days, but not in another trial of shorter duration. The added minerals increased the over-all incidence of diarrhea in the calves, whereas the animal tallow decreased its incidence in comparison to diets without added fat. The rather severe, apparently noninfectious diarrhea subsided after several days, even though the same amount of the respective diets was fed each day throughout the experiment, indicating a marked adaptation of the calves to diarrheic diets.

Addition of minerals to the diet appeared to accelerate, whereas added fat tended to delay, the rate of abomasal evacuation, as determined by radiography of 16 calves at four and 11 days of age. There was little evidence that the rate of abomasal evacuation was definitely related to the severity of diarrhea on the days the radiographs were made. The average weight, pH, and chemical composition of digestive tract contents in calves at 17 days of age were not related to fecal consistency at the time of slaughter.

The mineral content of various ingredients and the type of fat to use are among the factors that need to be considered in formulating milk replacers for dairy calves. Wing et al. (25) suggested that the high incidence of diarrhea observed in calves fed replacers containing a relatively large amount of dried whey or dried whey product (5, 23, 25, 26) was at least partially due to the high mineral content of these products. Further, Owen et al. (17) observed that the addition of minerals simulating whey ash to a skimmilk diet caused diarrhea in calves 11 days of age and older, whereas a lower incidence of diarrhea resulted when the minerals were added to whole milk containing 3.1% fat.

Different fats have been observed to vary greatly in nutritional value for young calves (2, 6, 9, 11, 14, 24). Part of this variation can be attributed to differences in digestibility of the fats (1, 6, 10, 18, 19); however, other factors are also known to be involved, since Raven and Robinson (18) found palm oil to have a lower protein-sparing effect than milk fat, even though the digestibility of the two fats was nearly equal. The nutritive value of some fats is increased by the addition of lecithin (10), and several fats are improved by homogenization (3, 10, 12) or hydrogenation (2, 14). In contrast, Raven and Robinson (18, 19) found unhydrogenated palm oil and palm-kernel oil to be superior to the corresponding hydrogenated products, as measured by their effect on the nitrogen retention of calves. Other factors affecting the nutritive value of milk replacers for calves have been discussed by Schuh (20).

The objective of the present study was to determine the effects of adding minerals simulating whey ash, animal tallow, or both, to a basal nonfat milk solids (NFMS) diet for young calves under conditions of continuous feeding.

EXPERIMENTAL PROCEDURE

In each of two separate trials, 16 male Holstein calves were assigned at random to treatment groups as follows: (a) basal NFMS diet, (b) basal diet plus fat, (c) basal diet plus minerals, (d) basal diet plus minerals and fat. All calves were started on experiment at three days of age as they became available over a period of several months. In Trial I,

Received for publication December 14, 1962.

[1] Taken from a thesis presented to the Graduate School, Oklahoma State University, by J. D. Schuh, in partial fulfillment of the requirements for the Ph.D. degree.

[2] Present address: Dairy Extension Specialist, University of Nevada, Reno.

extending from May to February, calves were used for radiographic studies and afterwards killed at 17 days of age. The calves in Trial II, extending from February to July, were maintained on the experimental rations until 24 days of age.

The basal diet contained 10% dried nonfat milk solids. Diets containing 3% animal tallow were prepared by homogenizing the tallow at 2,000 psi with the basal diet. The mineral supplement simulating whey ash (450 g $CaHPO_4 \cdot 2H_2O$, 360 g K_2HPO_4, 175 g $MgSO_4$, and 390 g NaCl) was added to the milk immediately before each feeding at the rate of 2.1% of the milk consumed. This level of minerals was used because it approximated the level in whey product reconstituted to have the same energy content as whole milk. Also, it appeared desirable to compare the effects of feeding this level of minerals to calves on a continuous basis starting at four days of age with results previously reported by Owen et al. (17) with older calves fed similar diets on an intermittent, short-term basis. Supplemental vitamins and trace minerals[3] were added to all the diets in amounts similar to those previously used by other workers (6, 13, 21), to insure that the results would not be confounded by deficiencies of these nutrients.

Each calf was fed colostrum for two days after birth, and thereafter the respective experimental diets were fed at 12-hr intervals at the daily rate of 8% of initial body weight. The calves were housed in a barn in which the temperature was controlled within the limits of 60-85 F. Individual pens having metal screen floors were used, and ratings on fecal consistency were made at frequent intervals (3. 6, and 9 AM; 2, 5, 8, and 10 PM) during each day, using a geometric scale of 1, 2, 4, and 8, denoting normal, very soft, semifluid, and extremely fluid feces, respectively. Body weights were recorded daily and blood samples were taken at four-day intervals for hematocrit evaluation, to detect any marked dehydration of the calves. The diarrhea which occurred was considered to be of nutritional origin as long as the calves remained alert, consumed the allotted amount of feed, and the rectal temperature remained below 103.5 F. This temperature was chosen because calves were frequently observed to have a body temperature this high with no other clinical evidence of infection under the prevailing climatic conditions.

Radiographs were made of the 16 calves in Trial I at four and 11 days of age, to determine the time required for abomasal evacuation and passage of the diets through the intestinal tract. After making a survey radiograph, barium sulfate (Barotrast[4]) was fed to each calf at the rate of 1 oz/pound of the usual diet. A series of radiographs was then made, beginning with one immediately after the test meal, and then at 0.5, 3, 9, 12, and 24 hr thereafter. Feeding of the calves was delayed at 12 and 24 hr until the radiographs were made.

The radiographs were made, using a Picker mobile machine, Kodak Royal Blue medical film, and 14- by 17-in. Picker Lysholm grid front cassettes with Patterson high-speed intensifying screens. The cassettes were placed in an upright cassette holder on the right side of the standing calf (Figure 3), with a focal-spot cassette distance of 30 in. The average exposure for a calf measuring 18 cm in the anterior lumbar area was 50 ma at 0.3 sec and 73 kv. As the calves varied from 16

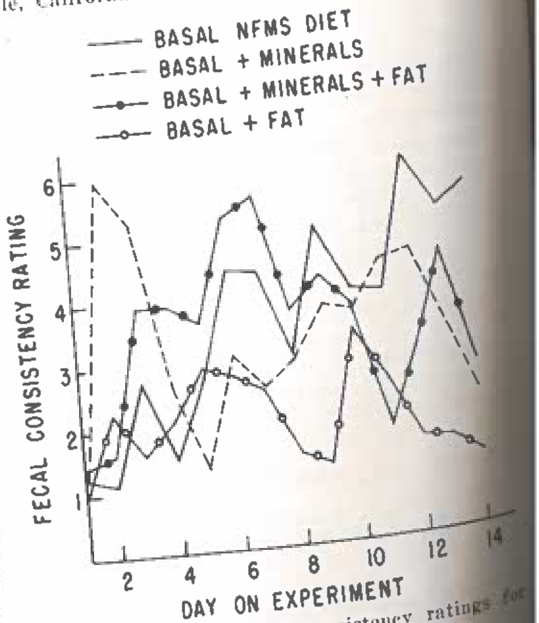


FIG. 1. Average fecal consistency ratings for calves fed experimental diets for 14 days.

---

[3] Diets supplemented with vitamins, trace minerals, and antibiotics as follows: Amounts per calf daily: 5,000 IU vitamin A, 625 IU vitamin D, 20 mg thiamine, 20 mg riboflavin, 10 mg pyridoxine, 10 mg Ca pantothenate, 20 mg niacin, 100 mg inositol, 0.2 mg biotin, 12 mg p-aminobenzoic acid, 20 mg alpha-D-tocopherol, 1 mg folic acid, 1 mg menadione, 0.01 mg vitamin B_{12}, 60 mg chlortetracycline. Amounts per pound of diet: 11.1 mg $FeSO_4$, 2.1 mg $CuSO_4$, 8.0 mg $MnSO_4 \cdot H_2O$, 2.5 mg bacitracin, 1.0 mg penicillin, 170 mg choline chloride.

[4] Micronized barium sulfate supplied by the Barnes-Hind Barium Products Company, Sunnyvale, California.

to 20 cm in thickness, the voltage was varied from 70 to 75 kv for the different-sized calves. The volume of ingesta in the abomasum at different periods was calculated from measurements on the radiographs, on the basis that the material approximated the shape of a prolate spheroid.

At 17 days of age each calf was killed 6 hr after feeding, and determinations were made of the weight and pH of the contents in the rumen-reticulum, omasum-abomasum, and the anterior and the posterior parts of the small and large intestines. Representative samples from each segment were analyzed for dry matter, ash, protein nitrogen, and crude fat, using AOAC methods (Official Methods of Analysis, 8th ed.), except that aliquots for ash and crude fat analyses were taken after the samples were dried to a constant weight under vacuum at 60 C.

In Trial II, calves were fed the experimental diets until 24 days of age, to provide additional time for possible adaptation to the high mineral diets. Carmine was administered to these calves by mixing with the milk immediately before feeding on the second, eighth, and fourteenth days of the experiment, to determine the interval required for passage of the respective diets through the digestive tract. Indigo carmine was used initially, but a change was made to carmine red because it was easier to see in the feces than was the other type.

Since the daily fecal consistency values represented an average of all the ratings made during each day, a normal distribution was assumed. The analysis of variance for each trial followed essentially that for a split plot in time, Sec. 12.5 of Steel and Torrie (22), with sources of variation of the main plot being treatments and calves within treatments (Error A); sources of variation for the subplots were days, days times treatments, and days times calves within treatments (Error B). The mean square for error for evaluating treatment-period interactions was the pooled value for animals within days (periods) by treatments, yet the degrees of freedom for determining statistical significance were that for a single period.

RESULTS AND DISCUSSION

In Trial I, the calves fed diets with added minerals had a higher over-all incidence of diarrhea than those fed diets without the added minerals, and the calves fed added fat had less diarrhea than those fed diets without added fat. However, neither these main effects nor the interaction of the two factors was statistically significant ($P > 0.05$) for the entire trial. On the second day of the experiment, when barium sulfate was administered for the purpose of taking the first series of radiographs, there was a significant interaction ($P < 0.005$) of the two factors, with a high incidence of diarrhea in the group fed the basal diet plus added minerals, but not in the group fed both added minerals and fat (Figure 1). These results were interpreted as evidence that administration of barium sulfate at four days of age had an adverse effect on the calves fed the basal diet with added minerals, but no immediate effect on calves fed the other diets. It is possible that the response of the calves in this trial was affected sufficiently by administration of the barium sulfate to the calves at four and 11 days of age to account for a lack of complete agreement of results in the two trials reported herein.

In Trial II, there was also a higher over-all incidence of diarrhea in calves fed the diets containing added minerals than in those fed the other diets, whereas the calves fed diets with added fat had less diarrhea than those fed diets without added fat. The independent effects of both minerals and fat were statistically significant ($P < 0.01$), but neither the interaction of these two factors nor the interaction of these factors with periods (days) was statistically significant ($P > 0.05$). The calves fed the basal diet plus added minerals had very severe diarrhea within one to two days after feeding of the experimental diets was begun. Equally severe, but less persistent, diarrhea was evident within five or six days in the group receiving the diet to which both minerals and fat had been added. Thus, the added fat did not prevent severe diarrhea in calves fed a high-mineral diet, although its duration was reduced. In a similar experiment, Owen et al. (17) observed that milk fat had an antidiarrheic action when added to diets containing added minerals; however, the latter workers used older calves (11 vs. three days of age) and an experimental design wherein the diets were tested during periods of only four days, with minimal three-day adjustment periods of whole milk feeding before subsequent test feedings.

There was a general increase in the incidence of diarrhea within a few days after the trial began and a decrease toward the latter part of the trial (Figure 2). These differences among days on the experiment were statistically significant ($P < 0.05$) and indicative of a rather marked adaptation of the calves to the diets following periods of severe diarrhea. Thus, in the absence of infection, the

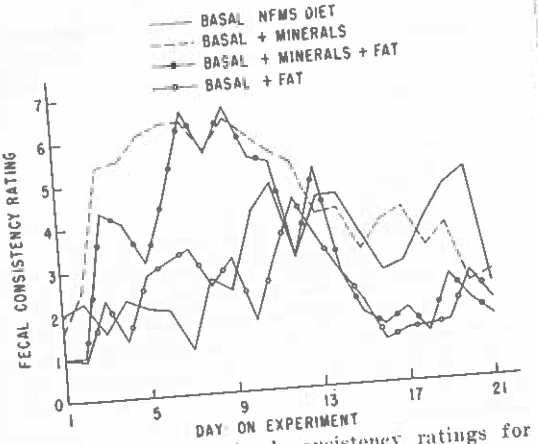


FIG. 2. Average fecal consistency ratings for calves fed experimental diets for 21 days.

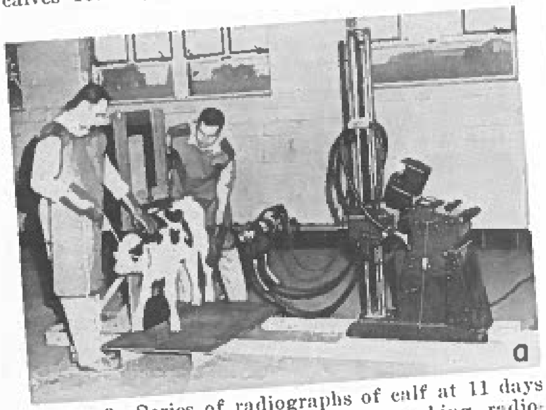
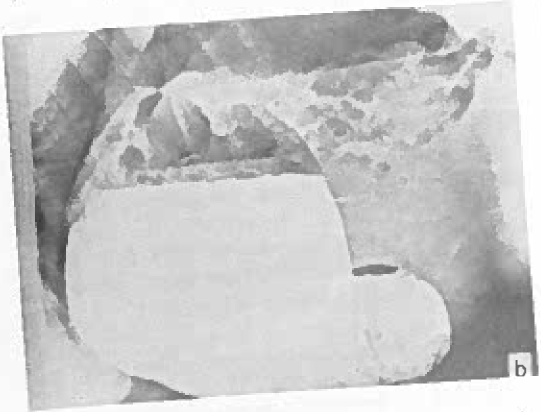
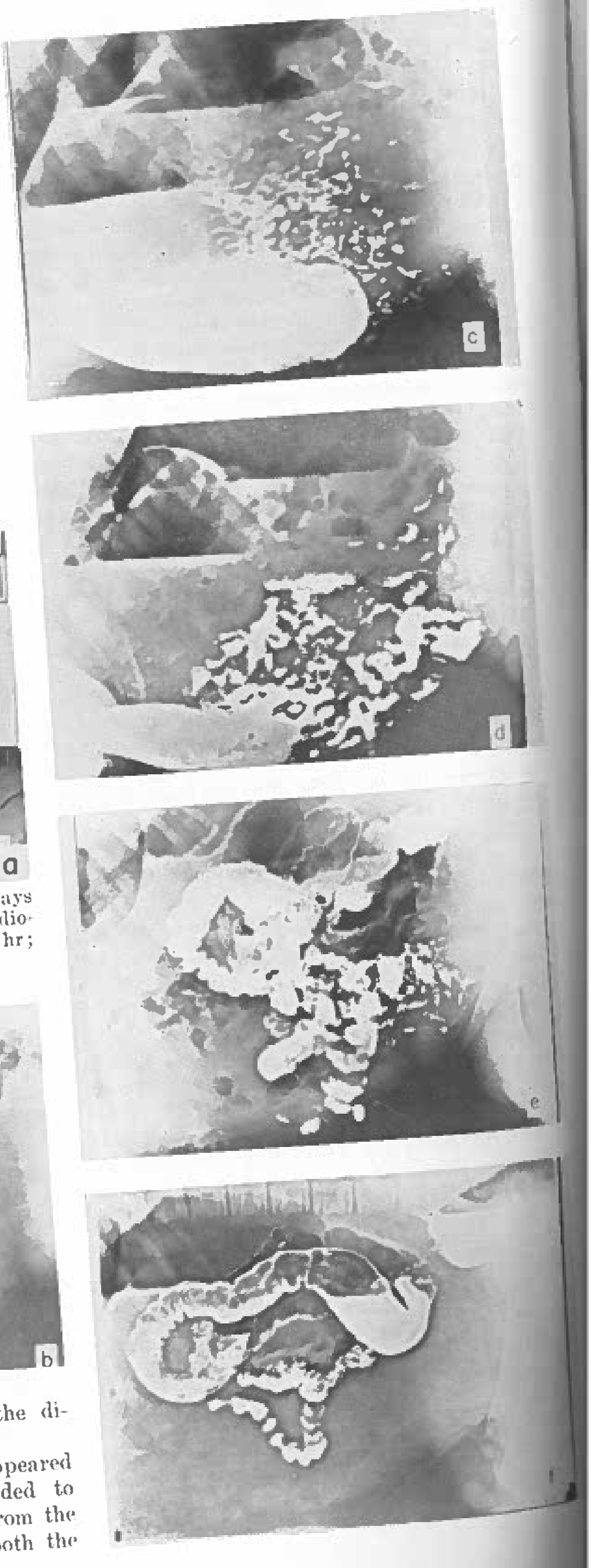


FIG. 3. Series of radiographs of calf at 11 days of age: a) position of calf for making radiographs; b) immediate; c) 30 min; d) 3 hr; e) 9 hr; f) 12 hr after feeding.





calves exhibited a high tolerance for the diarrheic diets.

The addition of minerals to the diet appeared to accelerate, whereas added fat tended to delay, the rate of removal of ingesta from the abomasum (Table 1). The effects of both the

TABLE 1

Removal of barium test diet from the abomasum of calves as determined by radiographs at specified intervals after feeding

Diet	Age	Amount of test diet	Fecal consistency rating[a]	Per cent of test diet remaining after following number of hours				Time required for removal of following percentage of test diet from abomasum	
				0.5	3	9	12	50	75
	(days)	(lb)		(%)				(hr)	
Basal NFMS	4	3.3	1.12	60	16	1	0	1.0	2.2
Basal and fat	4	3.5	2.25	71	15	5	5	1.2	2.0
Basal and minerals	4	3.3	5.98	30	14	4	2	0.4	1.2
Basal and fat and minerals	4	3.6	1.62	61	10	4	2	0.8	1.5
Basal NFMS	11	3.3	5.00	41	10	2	0	0.4	0.8
Basal and fat	11	3.5	1.25	53	10	1	1	0.6	1.2
Basal and minerals	11	3.3	3.79	26	11	1	1	0.3	0.7
Basal and fat and minerals	11	3.6	6.00	62	19	6	2	1.0	2.4

[a] Consistency rating on day radiographs were taken.

added minerals and fat on the per cent of initial contents remaining in the abomasum one-half hour after feeding were statistically significant ($P < 0.05$) at four days of age. Although the minerals and fat appeared to have a different effect when added in combination than when added singly, this interaction was not statistically significant ($P > 0.10$). There was little relation between the diet and the per cent of initial contents estimated to be in the abomasum at the other intervals when radiographs were made.

The addition of fat resulted in a significant decrease ($P < 0.01$) in the rate of abomasal evacuation at 11 days of age. The added minerals consistently increased the rate of removal when added singly, but appeared to have the opposite effect in some calves fed the diet with both minerals and fat. The interaction of the two factors was not statistically significant ($P > .10$), however, possibly because of the relatively small number of calves involved.

Inconclusive evidence was obtained regarding the relationship of the rate of abomasal evacuation to the fecal consistency ratings on the days the calves were radiographed. A statistically significant ($P < 0.05$) correlation coefficient of -0.53 was found between the fecal ratings at four days of age and the per cent of test diets remaining in the abomasum at one-half hour after feeding; however, a small, nonsignificant correlation of -0.03 was found when the number of hours required for removal of one-half of the ingesta was used as the criterion of abomasal evacuation. Moreover, the comparable correlation coefficients computed on data pertaining to the calves at 11 days of age were 0.17 and 0.29, respectively. These observations coincide with the results of other recent work (8, 16), wherein the prevention of curd formation in the calf's stomach by the addition of sodium citrate did not affect the growth or health of young dairy calves.

Some of the barium sulfate passed into the small intestine within a short time after feeding (Figure 3), and had traversed the digestive tract to the rectum of the calves by the time the 9-hr radiographs were made, in most instances. These results agreed with the observations of Benzie and Phillipson (4), that barium reached the large intestine of 4-wk-old calves by 3.5 hr after feeding. In the present experiment, 13 ± 6.5 hr (mean ± standard deviation) was required for the carmine to pass through the tract and appear in the feces of calves having fecal consistency ratings from 2.0 to 8.0, whereas it was not evident in the feces until 26 ± 13.6 hr after feeding in calves with fecal consistency ratings of 1.0-2.0. Thus, it was concluded that the time required for carmine to appear in the feces did not give an accurate measure of the rate of passage of diets through the digestive tract of calves with normal fecal consistency.

With the exception of one group of calves discussed above, there was no evidence that the micronized barium sulfate used in this study had any adverse effect on the calves, and it is certain that studies can be made on calves at an earlier age, using a radiographic technique, than would be feasible by other techniques involving palpation via a rumen fistula. Moreover, the determinations made of the time required for abomasal evacuation in this work substantiate the results obtained by other workers (7, 15) using a palpation technique.

Calves having diarrhea were often observed to have a considerable amount of fluid in the large intestine, causing marked distention, in contrast to the more typical situation in calves without diarrhea (Figure 4). Evidence of colitis was seen on the radiographs of several calves, but it was not related to any specific diet.

There were no differences among groups in chemical composition of the digestive tract contents associated with fecal consistency at the time of necropsy. However, the possibility that differences in the composition may have existed when the range in fecal consistency ratings was greater than at the time of sacrifice cannot be discounted, since adaptation to some diets had occurred by the time the animals were killed (Figure 1). Similarly, Owen (15) found no relation between the incidence of diarrhea in calves and the weight or pH of digestive tract contents 14 hr after receiving whole milk or high lactose-mineral diets.

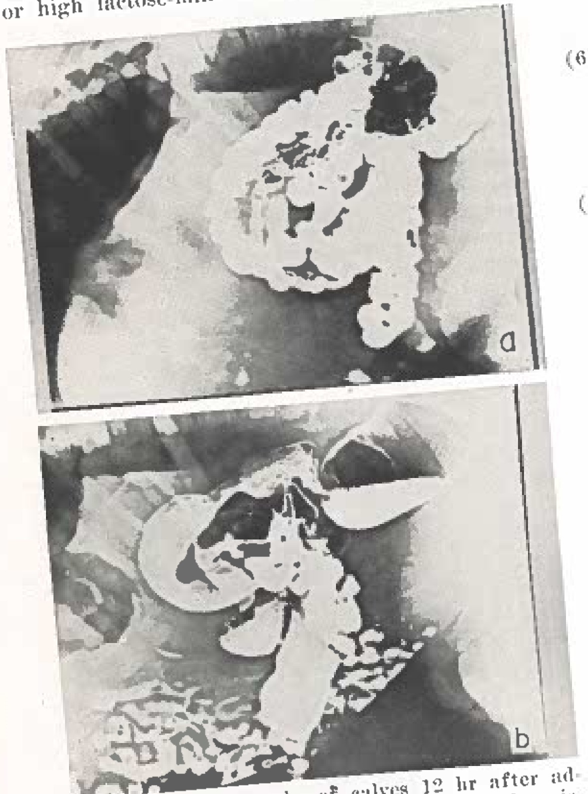


FIG. 4. Radiographs of calves 12 hr after administration of contrast media: a) typical peristalsis in calf having normal fecal consistency; b) marked distention and fluid level in large intestine of calf with diarrhea.

ACKNOWLEDGMENT

The help of E. W. Jones and R. J. Panciera in connection with certain phases of this work is gratefully acknowledged, as is the assistance of R. D. Morrison with the statistical analyses.

REFERENCES

(1) ADAMS, R. S., GANDER, J. E., GULLICKSON, T. W., AND SAUTTER, J. H. Some Effects of Feeding Various Filled Milks to Dairy Calves. II. Fecal Characteristics and Digestibility Data. J. Dairy Sci., 42: 1562. 1959.

(2) ADAMS, R. S., GULLICKSON, T. W., GANDER, J. E., AND SAUTTER, J. H. Some Effects of Feeding Various Filled Milks to Dairy Calves. I. Physical Condition and Weight Gains with Special Reference to Low-Fat Rations. J. Dairy Sci., 42: 1552. 1959.

(3) BATE, W., ESPE, D., AND CANNON, C. Y. Influence of Homogenization of Fat on Haircoat of Dairy Calves. J. Dairy Sci., 29: 41. 1946.

(4) BENZIE, D., AND PHILLIPSON, A. T. The Alimentary Tract of the Ruminant. Charles C Thomas, Publisher, Springfield, Ill. 1957.

(5) BROWN, D. C., READ, J., AND WILLARD, H. S. Slacked Lime as a Preventive of Scours for Whey-Fed Calves. J. Dairy Sci., 36: 319. 1953.

(6) CUNNINGHAM, H. M., AND LOOSLI, J. K. The Effect of Fat-Free Diets on Young Dairy Calves with Observations on Metabolic Fecal Fat and Digestion Coefficients for Lard and Hydrogenated Coconut Oil. J. Dairy Sci., 37: 453. 1954.

(7) DICKEY, H. C., ESPE, D., AND CANNON, C. Y. The Relation of the Rate of Disappearance of Milk Curd from the Calf's Abomasum to the Apparent Digestibility of Its Protein. J. Dairy Sci., 22: 464. 1939.

(8) FARHAN, S. M. Milk Replacer Studies with Dairy Calves. M.Sc. thesis, Oklahoma State University, Stillwater. 1962.

(9) GULLICKSON, T. W., FOUNTAINE, F. C., AND FITCH, J. B. Various Oils and Fats as Substitutes for Butterfat in the Ration of Young Dairy Calves. J. Dairy Sci., 25: 117. 1942.

(10) HOPKINS, D. T., WARNER, R. G., AND LOOSLI, J. K. Fat Digestibility by Dairy Calves. J. Dairy Sci., 42: 1815. 1959.

(11) JACOBSON, N. L., CANNON, C. Y., AND THOMAS, B. H. Filled Milks for Dairy Calves. I. Soybean Oil vs. Milk Fat. J. Dairy Sci., 32: 429. 1949.

(12) KASTALIC, J., BENTLEY, O. G., AND PHILLIPS, P. H. Studies on Growth and Survival of Calves Fed Semisynthetic Milks from Birth. J. Dairy Sci., 33: 725. 1950.

(13) MATRONE, G., RAMSEY, H. A., AND WISE, G. H. Purified Diets for Ruminants. Proc. Soc. Exptl. Biol. Med., 95: 731. 1957.

(14) MURLEY, W. R., JACOBSON, N. L., WISE,

G. H., AND ALLEN, R. S. Filled Milks for Dairy Calves. II. Comparative Effects of Various Types of Soybean Oils and of Butter Oil on Health, Growth, and Certain Blood Constituents. J. Dairy Sci., 32: 602. 1949.

(15) OWEN, F. G. Interrelationships of Lactose, Lipids and Minerals in Nutrient Utilization by the Calf. Ph.D. thesis, Iowa State University, Ames. 1956.

(16) OWEN, F. G., AND BROWN, C. J. Interrelationships of Milk Temperature, Dilution, and Curd Formation in the Response of Calves to Whole Milk Diets. J. Dairy Sci., 41: 1534. 1958.

(17) OWEN, F. G., JACOBSON, N. L., ALLEN, R. S., AND HOMEYER, P. G. Nutritional Factors in Calf Diarrhea. J. Dairy Sci., 41: 662. 1958.

(18) RAVEN, A. M., AND ROBINSON, K. L. Studies of the Nutrition of the Young Calf. 2. The Nutritive Value of Unhydrogenated Palm Oil, Unhydrogenated Palm-Kernel Oil, and Butterfat, as Additions to a Milk Diet. Brit. J. Nutrition, 13: 178. 1959.

(19) RAVEN, A. M., AND ROBINSON, K. L. Studies of the Nutrition of the Young Calf. 3. A Comparison of Unhydrogenated Palm-Kernel Oil, Hydrogenated Palm-Kernel Oil, and Butterfat, as Constituents of a Milk Diet. Brit. J. Nutrition, 14: 135. 1960.

(20) SCHUH, J. D. Nutritional Factors in Calf Diarrhea. Ph.D. thesis, Oklahoma State University, Stillwater. 1960.

(21) SHEPPARD, A. J., AND JOHNSON, B. C. Pantothenic Acid Deficiency in the Growing Calf. J. Nutrition, 61: 195. 1957.

(22) STEEL, R. G. D., AND TORRIE, J. H. Principles and Procedures of Statistics. McGraw-Hill Book Company, Inc., New York. 1960.

(23) WALLACE, H. D., LOOSLI, J. K., AND TURK, K. L. Substitutes for Fluid Milk in Feeding Dairy Calves. J. Dairy Sci., 34: 256. 1951.

(24) WIESE, A. C., JOHNSON, B. C., MITCHELL, H. H., AND NEVENS, W. B. Synthetic Rations for the Dairy Calf. J. Dairy Sci., 30: 87. 1947.

(25) WING, J. M., JACOBSON, N. L., AND ALLEN, R. S. The Effect of Various Restricted Diets on the Growth and on Certain Blood Components of Young Dairy Calves. J. Dairy Sci., 38: 1006. 1955.

(26) YOUNG, F. B. Response of Dairy Calves to Whole Milk Replacements Containing Dried Whey Product, Dried Skimmilk, and Fat. J. Dairy Sci., 36: 600. 1953.

RESPONSES OF DAIRY CALVES TO MILK REPLACER FED AT VARIOUS CONCENTRATIONS

J. D. PETTYJOHN, J. P. EVERETT, JR., AND R. D. MOCHRIE
Animal Science Department, North Carolina Agricultural Experiment Station, Raleigh[1]

SUMMARY

This study was undertaken to determine the effects of nutrient concentration on intake and growth by the calf. Thirty male Holstein calves 14 days old were divided among five treatments, consisting of ad libitum feeding of a commercial milk replacer mixed with water to contain 5, 10, 15, 20, or 25% dry matter. Digestibility and nitrogen retention were determined following the growth trial.

Feed intakes increased and dry matter intakes decreased as dry matter in the diet decreased. Calves fed the 15% diet gained faster, increased more in height at withers, had equal or better efficiencies of nutrient utilization and higher veal grades than calves fed other concentrations. Utilization of dry matter was reduced markedly at higher concentrations. Dry matter and crude protein digestibilities decreased with increasing concentration. Intake of water per se increased with increasing concentration of dry matter, whereas total water decreased.

Feed and dry matter intakes indicate that at the 5% dry matter concentration the physical capacity of the calves limited the amount of feed eaten. At the higher nutrient concentrations, the calf attempts to limit its level of nutrient intake.

Factors affecting the feed intake of ruminants are diverse and difficult to measure. Capacity of the tract and the rate of passage of undigested material through the tract are two factors that could limit the total amount of a feed eaten. However, when the concentration of nutrients in the feed is high enough to preclude a capacity limitation, does feed intake decrease, increase, or remain constant with increasing nutrient concentration? For simple-stomached animals the evidence indicates that a constant caloric intake is effected when rations of different caloric density are fed. Studies with rats (1, 4, 10) and dogs (3, 4) have shown that dilution of a ration elicits a nearly proportional increase in total intake with only slight modification of caloric intake. Results obtained from either increasing the caloric density of a diet (10) or intragastric feeding (5) support observations from dilution studies.

This trial was undertaken to study the effects of dietary nutrient concentration and, indirectly, gastrointestinal capacity on the nutrient intake of dairy calves. If consumption during ad libitum feeding is primarily controlled by physical capacity, nutrient intake would be in direct proportion to nutrient concentration, but if caloric intake is the only controlling factor, then nutrient intake should be similar over a wide range of concentrations. A preliminary report of this work appeared earlier (7).

EXPERIMENTAL PROCEDURE

Thirty male Holstein calves at three days of age were transferred from their dams to individual stalls in a conventional calf barn. The calves were fed whole milk twice daily at a rate of 8% of body weight until seven days of age and a milk replacer diet (13% dry matter) at the same rate through the 14th day. Each calf was randomly assigned to one of five dietary treatments, consisting of the milk replacer mixed with water to contain 5, 10, 15, 20, or 25% dry matter and being fed from 15 through 56 days of age. To avoid a sudden change in the dietary of each calf, the total amount of dry matter fed on the 15th day approximately equaled that for the 14th day. Thereafter, the amount of dry matter was increased by 0.3 to 0.5 lb over the previous day until ad libitum intake was reached, as indicated by a 10 to 15% refusal daily. The replacer was fed at approximately 100 F in four equal feedings at 6-hr intervals via nipple

Received for publication March 27, 1963.

[1] Contribution from the North Carolina Agricultural Experiment Station, Raleigh, North Carolina. Published with the approval of the Director of Research as Paper No. 1586 of the Journal Series.

pails. Drinking water was available at all times. The dry milk replacer was weighed to the nearest 0.01 lb and the mixed replacer and refusals to the nearest 0.1 lb at each feeding.

The calves were weighed at three and seven days of age and at weekly intervals thereafter, all weights being recorded at 8 AM. Height at withers was recorded at seven and 14 days of age and at 2-wk intervals afterwards. Incidence of scours was recorded twice daily. The amount of water consumed in addition to that in the replacer was measured during the last week each calf was on trial. The dry replacer was sampled weekly and then composited by lots for analyses. Four different lots of replacer were used and had an average percentage composition of 95.92 dry matter, 7.93 ash, 28.10 crude protein, 10.10 ether extract, 0.73 crude fiber, and 49.06 nitrogen-free extract. Since ad libitum intake was not attained until the second week of the comparison period, data from 29-56 days only were used for feed intake and growth comparisons.

Following the growth study, each calf was transferred to a digestion crate for a four-day urine and fecal collection to determine dry matter and crude protein digestibilities and nitrogen retention. After the digestion trial, each calf was slaughtered and dressed carcass weight and carcass grade determined.

RESULTS

Mean values for the various intake data, together with the significance of the trend analyses, are given in Table 1.

As the concentration of dry matter increased, the consumption of liquid milk replacer declined at a decreasing rate. This relationship was also evident when the as-fed intakes were calculated per 100 lb of liveweight.

The patterns of intakes of dry matter and of dry matter per 100 lb of liveweight were similar. The lower dry matter intakes at the lower concentrations of replacer suggest that the physical capacity of the gastrointestinal tract limited nutrient intake. The relatively large difference between the dry matter intakes for calves fed the 15, 20, and 25% diets and the intakes of calves fed the 5 and 10% concentrations emphasizes the role of physical capacity.

The mean animal response data and efficiency of feed utilization are given in Table 1. Trends for increase in height and for average daily gain have a definite curvilinear pattern. As the concentration of milk replacer increased, growth, as measured by increased height and weight, also increased until the dry matter concentration reached 15%. The relative responses at the lower concentrations are to be expected from the relative nutrient intakes on

TABLE 1

Average daily feed and water[a] intakes, growth responses, dry matter utilization, and incidence of scouring (29-56 days of age)

Criterion	Per cent dry matter in replacer					Standard error of a mean	Significance of trends	
	5	10	15	20	25		Linear	Quadratic
							(P <)	
Average daily intake								
As fed *(lb)*	59.4	39.2	29.8	23.2	19.4	2.97	0.001	0.05
As fed per 100 lb live weight *(lb)*	38.0	23.6	18.5	14.2	12.5	1.34	0.001	0.001
Dry matter *(lb)*	3.06	4.02	4.60	4.79	4.90	0.29	0.001	0.1
Dry matter per 100 lb live weight *(lb)*	1.96	2.42	2.86	2.92	3.16	0.12	0.001	0.1
Average daily intake of water, per se *(lb)*	0.9	3.4	5.1	8.4	11.6	2.28	0.01	NS[b]
Average daily total water intake *(lb)*	57.2	37.6	29.3	23.9	22.6	3.54	0.001	0.05
Response								
Increase in height *(in.)*	2.12	2.23	2.73	2.21	2.08	0.17	NS	0.05
Average daily gain *(lb)*	1.57	2.09	2.42	1.85	1.96	0.20	NS	0.05
Dry matter per lb of gain *(lb)*	2.04	1.89	1.92	2.82	2.83	0.27	0.01	NS
Days with moderate or severe scours	1.0	1.2	5.0	6.2	5.8	1.92	0.05	NS

[a] Water intakes were measured from 50-56 days only.

[b] NS—Nonsignificant.

these treatments. At the higher concentrations, even though the nutrient intakes were slightly higher than for calves fed the 15% concentration, growth was somewhat less. This poorer growth response can only be explained by a reduced utilization of ingested nutrients. Janowitz and Hollander (5) and Share et al. (9) noted that when more than 100% of the control intake in dogs was fed intragastrically, the body weight gain of these dogs was much less than expected.

The increasing amount of dry matter required per pound of gain as concentration of dry matter increased verifies reduced utilization as a cause of the poorer growth response of calves fed the 20 and 25% dry matter concentrations. The calves fed the 5, 10, and 15% levels had very similar efficiencies, which were more favorable than those for calves fed the 20 and 25% levels.

The occurrence of moderate or severe scouring during the growth trial (Table 1) appears to offer a partial explanation for the inefficiency of the calves fed the 20 and 25% concentrations, since they had the greatest incidence. However, the calves fed the 15% level of dry matter had only one day less with scours and yet they also had an average efficiency similar to the calves with the lowest incidence of scouring.

The mean intakes for water drunk free choice and total water intake during the last week on trial are presented in Table 1. The trend for daily intake of water, per se, was linear, with the amount of water imbibed increasing as the concentration of dry matter in the milk replacer increased. The total water intake decreased as nutrient concentration increased, but tended to level off at the higher dry matter concentrations. The dry matter intakes expressed as a percentage of the total water for this period were 5.4, 10.5, 15.2, 17.9, and 18.5. Although these concentrations are somewhat different, except for the lowest values, they are in the range expected for ruminants (11) and would probably not account for the observed differences in efficiency of feed utilization.

Data from the digestion and balance trial (Table 2) show that dry matter digestibility had a negatively linear trend, decreasing as nutrient concentration increased, but appeared to level off at the higher concentrations.

Crude protein digestibility followed the same trend, with values for the three higher concentrations being similar but all lower than those for the two lowest concentrations. Nitrogen retention was highly variable and showed no definite pattern.

The mean digestibility percentages for the appropriate concentrations were applied to the individual intakes in the growth trial to give estimates of digestible dry matter intake (Table 3). These values were curvilinear with respect to concentration, as was the trend in gross dry matter intakes. Utilization of this digestible dry matter followed a pattern similar to that for total dry matter, with the pounds of digestible dry matter required for a pound of gain decreasing as nutrient concentration increased to the 15% level, then increasing for the two highest levels.

The average pounds of TDN required per pound of gain (Table 3) showed a positive curvilinear trend very much like that for digestible dry matter utilization. The efficiency of TDN utilization decreased as dry matter concentration in the ration increased, but leveled off at the 20 and 25% concentrations.

Carcass grades (Table 3) show a trend similar to average daily gain, with the calves fed the 10 and 15% concentrations having the highest average grades. This is also in line with feed efficiency.

TABLE 2

Dry matter digestibility, crude protein digestibility, and nitrogen retention

Criterion	Per cent dry matter in replacer					Standard error of a mean	Significance of trends	
	5	10	15	20	25		Linear	Quadratic
							(P <)	
Dry matter digestibility (%)	85.5	82.4	79.4	78.8	79.1	2.37	0.05	NS[a]
Crude protein digestibility (%)	74.6	69.5	60.5	62.0	62.8	4.04	0.05	NS[a]
Nitrogen retention[a] (g/day)	20.9	16.7	23.6	30.2	25.3	4.62	0.1	0.1

[a] Nonsignificant.

TABLE 3

Digestible dry matter intake and utilization, TDN utilization (29-56 days), and carcass grades

Criterion	Per cent dry matter in replacer 5	10	15	20	25	Standard error of a mean	Significance of trends Linear	Quadratic
							(*P* <)	
Digestible dry matter intake *(lb)*	2.62	3.37	3.73	3.90	3.82	0.22	0.001	0.05
Digestible dry matter per pound of gain *(lb)*	1.76	1.61	1.55	2.28	2.20	0.22	0.1	0.05
TDN per pound of of gain [a] *(lb)*	0.70	0.90	1.07	1.61	1.58	0.16	0.001	0.05
Carcass grade [b]	4.0	5.6	6.2	4.6	5.2	0.54	NS [c]	0.1

[a] Calculated after deducting maintenance requirements according to Brody (2). (See Table 15.1, p. 478.)

[b] Dressed carcasses were scored on the following scheme: cull, 0-2; utility, 3-5; standard, 6-8.

[c] Nonsignificant.

DISCUSSION

The increased intake volumes of the calves fed the 5 and 10% dry matter concentrations show that as nutrient concentration is reduced the calf tries to compensate by eating a greater volume. The rapid leveling off of the dry matter intakes at the higher concentrations, viz., the 15, 20, and 25% levels, also supports the view that nutrient (caloric) density is a factor influencing feed intake. These results are similar to observations on rats (1, 4, 10) and dogs (3).

The slight increase in dry matter intake per 100 lb liveweight as concentration of dry matter in the ration increased from 15 to 25% indicates that fill (distention of the gastrointestinal tract) may partially regulate nutrient intake. If fill were the only factor regulating intake within this range, however, the as-fed intakes per 100 lb liveweight would have been the same. Therefore, it seems that there is an interaction between a desire for a certain fill and the physiological desire for a certain caloric intake.

The fact that the calves fed the 20 and 25% dry matter concentrations had higher dry matter intakes, yet did not match the growth of the calves fed the 15% concentration (resulting in excessive values for TDN/lb of gain), is difficult to explain. Based on fecal collection after 56 days of age, it cannot be completely accounted for by normal fecal loss or incidence of scouring, but might possibly be similar to a phenomenon noted by Reid (8) and McClymont (6) in connection with acetate metabolism, where an excess of acetate led to oxidation with large energy losses.

Whether the results of this study can be applied to ruminants is indeed a nebulous question, but may be worth asking. Although the calves were not ruminants, there were indications (e.g., cud chewing) that they were in the transition stage of becoming ruminants. It might then be supposed that their physiological satiety mechanism would be similar to that for adult ruminants. Also, the apparent difference between the rations fed the calves and normal rations for adult ruminants may be only superficial, since a high concentrate ration has some similarity to the milk replacer fed at the higher concentrations and the lower concentrations might be compared with lush pasture. Consideration of these ideas makes it seem possible that ruminants, as well as nonruminants, strive to attain a certain caloric intake on low-energy diets and become satiated before limitations of fill occur on high-energy diets.

ACKNOWLEDGMENTS

The Hi-Fat CMR used in this study was supplied by the Borden Company, 350 Madison Avenue, New York, New York.

REFERENCES

(1) Adolph, E. F. Urges to Eat and Drink in Rats. Am. J. Physiol., 151: 110. 1947.

(2) Brody, S. Bioenergetics and Growth. Reinhold Publishing Corporation, New York. 1945.

(3) Grossman, M. I. Satiety Signals. Am. J. Clin. Nutrition, 8: 562. 1960.

(4) Janowitz, H. D., and Grossman, M. I. Effect of Variations in Nutritive Density on Intake of Food of Dogs and Rats. Am. J. Physiol., 158: 184. 1949.

(5) Janowitz, H. D., and Hollander, F. The Time Factor in the Adjustment of Food Intake to Varied Calorie Requirement in the Dog. A Study of the Precision of Appetite Regulation. Ann. N. Y. Acad. Sci., 63: 56. 1955.

(6) McClymont, G. L. Specific Dynamic Action of Acetic Acid and Heat Increment of Feeding in Ruminants. Australian J. Sci. Research (Series B), 5: 374. 1952.

(7) Pettyjohn, J. D., Everett, J. P., Jr., and Mochrie, R. D. The Effect of Dietary Dilution on Nutrient Intake by Dairy Calves. J. Dairy Sci., 44: 1183. 1961.

(8) Reid, R. L. Studies on the Carbohydrate Metabolism of Sheep. II. The Uptake by the Tissues of Glucose and Acetic Acid from the Peripheral Circulation. Australian J. Research, 1: 338. 1950.

(9) Share, I., Martyniuk, E., and Grossman, M. I. Effect of Prolonged Intragastric Feeding on Oral Food Intake in Dogs. Am. J. Physiol., 169: 229. 1952.

(10) Strominger, J. L., Brobeck, J. R., and Cort, R. L. Regulation of Food Intake in Normal Rats and in Rats with Hypothalamic Hyperphagia. Yale J. Biol. Med., 26: 55. 1953.

(11) Winchester, C. F., and Morris, M. J. Water Intake Rates of Cattle. J. Animal Sci., 15: 722. 1956.

ZINC REQUIREMENT OF HOLSTEIN BULL CALVES TO NINE MONTHS OF AGE [1]

W. J. MILLER, C. M. CLIFTON, AND N. W. CAMERON
Dairy Department, University of Georgia, Athens

SUMMARY

Fifteen Holstein bull calves were fed a semipractical type diet containing 8.6 ppm of zinc on an as-fed basis until 29 wk of age. Following this depletion phase, they were fed the same basal diet with the addition of various levels of zinc for 8 wk. As indicated by weight gains, feed consumption, testicle size and weight, testicle zinc content, blood zinc content, blood carbonic anhydrase activity, and clinical examination, calves fed the basal ration performed as well as those fed supplemental zinc. It was concluded that, under the conditions of this experiment, calves do not require more than 8.6 ppm of zinc in their diet for normal performance to nine months of age.

Severe zinc deficiency symptoms developed by 12 wk of age in Holstein bull calves fed a purified diet containing 3.6 ppm zinc (8). These deficient animals responded very quickly to supplemental dietary zinc (8). In another study, calves fed a practical diet containing 25 ppm zinc performed as well for 15 wk as those fed the same diet supplemented with zinc (9). Thus, it appeared that the zinc requirement for normal growth of calves was well above 3.6 ppm, but not substantially above 25 ppm.

It was suggested that a period much longer than 15 wk, a depletion phase, and a basal diet appreciably lower than 25 ppm zinc might be useful in further delineating the zinc requirement of cattle (9). The diet containing 3.6 ppm zinc which was used to develop and study zinc deficiency in calves (7, 8) was very expensive. Thus, it was reasoned that formulation of a ration which is relatively inexpensive, deficient in zinc, and which gives good performance when supplemented with zinc would be very helpful in zinc nutrition research. From analyses of various ingredients (10) and preliminary growth tests with calves a semipractical, reasonably economical diet was formulated containing 8.6 ppm zinc. This ration gave good performance when fed to calves.

The purpose of this study was to further define the zinc requirement of calves and especially to determine whether the amount needed was above or below the level of zinc in the special semipractical basal ration (zinc content of 8.6 ppm).

EXPERIMENTAL PROCEDURE

Fifteen Holstein bull calves were utilized in an experiment which consisted of a depletion phase (3rd—29th wk of age) and a treatment period of 8 wk duration immediately following the depletion phase. Throughout the study special care was taken to make sure that the animals did not obtain zinc from extraneous sources.

The calves were given 7 lb of whole milk per day until 39 days of age. From the 3rd through the 29th wk of age they were fed, ad libitum, a basal ration especially formulated to contain a low level of zinc. This diet contained 8.6 ppm zinc on an as-fed basis and had the following percentage composition: dried beet pulp, 90.2; glucose monohydrate (cerelose), 5.0; urea feed mixture (42% N), 1.5; dicalcium phosphate (food grade, anhydrous dibasic calcium phosphate), 2.0; sodium chloride, 1.0; sodium sulfate, 0.25; and the following in grams per 100 lb: vitamin A mixture, (250,000 IU/g), 2.0; vitamin D, (200,000 IU/g), 1.0; oxytetracycline HCl (Terramycin HCl), 5.0; oleandomycin (25%), 2.0; $MnSO_4 \cdot H_2O$, 3.2; $Fe_2(SO_4)_3 \cdot nH_2O$ [78.5% $Fe_2(SO_4)_3$ by assay], 2.0; $CuSO_4 \cdot 5\ H_2O$, 0.02; and KI, 0.05. On a dry matter basis, the diet had the following composition: crude protein, 13.0%; calcium 1.24%; phosphorus, 0.40%; and zinc, 9.6 ppm.

At the beginning of their 30th wk of age the animals were randomly assigned to one of four experimental rations which were fed ad libitum for 8 wk. The basal diet was used as a control. The others were identical except for the addition of; a) 0.56 g ZnO (10 ppm zinc), b) 1.69 g ZnO (30 ppm), and c) 3.94 g ZnO (70 ppm) per 100 lb of feed. To more effec-

Received for publication April 3, 1963.

[1] Journal Paper No. 284 of the College Experiment Station, University of Georgia.

tively determine whether the zinc requirement was above or below the level of the basal diet, a larger number of animals was placed on this treatment. Thus, five calves were randomly assigned to the basal diet, three each to the rations containing the intermediate levels of zinc and four to the one containing 70 ppm of added zinc.

In previous research it was shown that zinc-deficient calves consumed less feed, gained less weight, had lower blood zinc and carbonic anhydrase activity and had appreciably smaller testicles (8). They developed severe skin lesions and various other characteristic abnormalities recognizable by clinical examination (8). These measures were chosen as indicators of whether the zinc nutrition was adequate for normal performance of the animals. Because of the influence of a zinc deficiency on testicle development (6) and the ease of obtaining samples, a cross section of the testicles was chosen as an indicator of the effect of the treatments on zinc content in tissues.

Body weights were taken every 2 wk with three-day weights being collected at the beginning and end of the depletion and treatment phases. Blood samples were combined for the second and fourth, 14th and 16th, 27th and 29th, and the 35th and 37th wk of age for each animal. These were frozen until analyzed for zinc and carbonic anhydrase activity by procedures previously described (12, 13, 21). All samples were analyzed at least twice, to reduce the error due to determinational variability in these measures. Feed samples were taken at each mixing and analyzed for zinc (21).

At the end of each of the phases of the study the testicles were measured as described previously (8). Clinical examinations were made at frequent intervals for any indication of abnormalities. When the last phase was completed the bulls were castrated and the testicles trimmed to a kidney shape and weighed. They were then frozen in plastic bags until analyzed. The frozen testicles were cut crosswise, the visceral layer and the tunica albuginea removed, and cross sections of the remaining tissues analyzed for dry matter, zinc (21), and protein. Dry matter content was determined by drying in an oven at 75 C for one day followed by 95 C until dry. Protein was determined by using N (Kjeldahl) times 6.25.

RESULTS AND DISCUSSION

Clinical examinations did not reveal any indication of a zinc deficiency in any of the calves during the study. In the previous studies in which zinc deficiencies were produced each calf exhibited many skin and mouth lesions as well as an unthrifty appearance, stiff joints, and other abnormalities (7, 8).

The data on growth and feed consumption are presented in Table 1. There were no significant differences in weight gain and feed consumption between calves fed the control diet and those fed supplemental zinc. Those fed the control diet gained 0.18 lb per day more than the average of all the others during the 27-wk depletion period and 0.25 lb per day more during the 8-wk treatment period. Certainly, there would be no reason for supplemental zinc to have a detrimental effect at the levels used. On the other hand, the fact that the averages were higher for those fed the unsupplemented diet strongly supports the idea that even with a much larger number of animals there is very little probability that the supplemented groups would have gained sig-

TABLE 1

Weight gains and feed consumption of Holstein bulls fed a low-zinc diet from 3-29 wk of age and the same diet supplemented with various levels of zinc from 30-37 wk

		Depletion period 3-29 wk			Supplemental period 30-37 wk				
Group	No. animals	Zinc in diet	Wt gain	Feed consumption	Zinc in diet	Wt gain (actual)	Feed consumption	Wt gain (adjusted)	Feed consumption (adjusted)
		(ppm)	(lb/calf/day)		(ppm)	(lb/calf/day)			
1	5	8.6	1.78	6.1	8.6	2.57	12.7	2.56	12.6
2	3	8.6	1.56	5.6	18.4	2.34	12.2	2.35	12.6
3	3	8.6	1.52	5.4	38.1	2.17	10.3	2.19	11.0
4	4	8.6	1.70	6.6	78.6	2.43	12.4	2.42	11.7
Avg of groups 2, 3, and 4		8.6	1.60	6.0		2.32	11.7	2.33	11.8
S_d[a]			0.20	0.7		0.28	1.0	0.29	0.7

[a] S_d—Standard error of mean difference between Group 1 and Groups 2, 3, and 4 combined.

nificantly faster. In view of the very drastic effect which a zinc deficiency had on growth and feed consumption in the previous work (7, 8), it is reasonable to conclude that the level of zinc was not a limiting factor in the growth of the animals in this experiment.

Average daily gains during the depletion and supplemental periods were approximately 82 and 112% of Beltsville growth standards for Holstein bulls of comparable ages (5). Therefore, it appears that the basal diet was capable of supporting approximately normal growth with perhaps moderate improvements to be desired, especially during the first few months of life.

There was a very low correlation ($r = 0.11$) between the growth of the animals during the depletion phase and that during the supplemental period. Also, the within treatment regression coefficient of 0.16 lb of gain increase during the supplemental period for each 1 lb additional gain in the supplemental period was quite low. Thus, the use of gain during the first period as a covariate did not improve the efficiency of measuring gain in the later period. In contrast, the correlation between feed consumption during the two periods was 0.73 ($P < 0.01$). On a within treatment basis, feed consumption during the second phase increased 1.13 lb for each pound increase of the corresponding value during the first phase. These data are reflected in a lower standard error for the adjusted feed consumption than for the unadjusted values. Thus, use of the covariate improved the efficiency of measuring feed consumption.

There were no significant differences in blood zinc content or carbonic anhydrase activity between calves fed the basal diet and those given supplemental zinc (Table 2). The within treatment correlation between blood zinc at the end of the depletion phase and the same measure at the end of the supplemental period was quite low ($r = 0.14$). Thus, use of the data from the depletion phase as a covariate did not materially improve the precision with which treatment effects could be measured. The correlation coefficient between carbonic anhydrase activity determined for the same two periods was 0.55 ($P < 0.05$). The corresponding regression coefficient was 0.30.

The within treatment and periods correlation between whole blood zinc and carbonic anhydrase activity was 0.54 ($P < 0.01$). Assuming blood zinc to be the independent variable, the regression coefficient indicated that carbonic anhydrase activity increased 154 units for each 1 ppm increase in blood zinc. Averaging the data for each calf for the three measurement times during the depletion period before calculating the correlation between blood zinc and carbonic anhydrase activity resulted in a coefficient of 0.70 ($P < 0.01$). It is well established that zinc is an essential constituent of carbonic anhydrase (19). In a study in which some calves were zinc-deficient and others normal the simple correlation between blood zinc and carbonic anhydrase activity was 0.80 (8). However, a consistently high correlation has not been observed between these two constituents of the blood in other species (19, 20). Our knowledge of the factors which affect the zinc content in the blood of cattle is very scant. It is very interesting that the carbonic anhydrase was much higher for all animals than values observed previously in our laboratory by the same procedure (8). The only known

TABLE 2

Blood zinc and carbonic anhydrase activity of Holstein bulls fed a low-zinc diet from 3-29 wk of age and the same diet supplemented with various levels of zinc from 30-37 wk

		Blood zinc					Carbonic anhydrase activity				
		Depletion period			Treatment period Wk 35-37		Depletion period			Treatment period Wk 35-37	
Group	No. animals	Wk 2-4	Wk 14-16	Wk 27-29	Actual	Adjusted	Wk 2-4	Wk 14-16	Wk 27-29	Actual	Adjusted
		(ppm)					*(enzyme units/ml)*				
1	5	2.42	2.51	2.41	2.08	2.06	902	874	895	810	798
2	3	2.16	2.38	2.26	1.91	1.91	1,003	785	975	743	715
3	3	2.75	2.18	2.11	2.38	2.40	1,018	849	805	698	704
4	4	2.44	2.24	2.13	2.26	2.27	974	673	683	743	774
Avg 2, 3, and 4		2.45	2.26	2.16	2.19	2.20	996	759	807	729	735
S_d[a]				0.31	0.20	0.20			102	48	44

[a] S_d—Standard error of mean difference between Group 1 and Groups 2, 3, and 4 combined.

difference that might account for this is the change in ration.

The level of zinc in the diet had no significant effect on the size of the testicles as measured by the greatest diameter × the length (Table 3). The within treatment correlation between measurements taken at the end of the depletion period and at the end of the treatment phase was 0.67 ($P < 0.05$). The within treatment regression indicated that for each increase in this measurement of 1 in. at the end of the depletion phase the diameter × length was 0.80 in. larger at the end of the treatment phase. The corresponding over-all regression was 0.79. These measures indicate that using the measurements at the end of the depletion phase as covariate increased the precision with which treatment effect on testicle size could be measured. None of the diets had any significant influence on the weight of the testicles at the end of the experiment. The amount of zinc in the testicles was not affected by the addition of zinc to the diet (Table 3). In an earlier study it was shown that all the tissues analyzed were lower in zinc-deficient than in normal calves (8). Since the testicles are one of the tissues which appears to be most drastically affected by zinc deficiency it seemed reasonable to use it as an indicator of any changes which might have occurred in animals fed the diets with and without supplemental zinc. In rats zinc deficiency produced atrophy of the testes and a reduction of zinc content (6). The authors are not aware of previous studies in which the zinc content of bovine testicles has been studied.

Vallee has suggested that expressing zinc content on a zinc/nitrogen basis is a more desirable basis of comparison for relating composition to function, because this metal is usually associated with protein (20). Thus, the zinc content of testicles is expressed on a fresh basis, a dry basis, and a protein (N × 6.25) basis (Table 3). The dry matter and protein were not affected by the supplemental zinc. The average zinc values are very close for the various groups of animals, regardless of the method of expression. The coefficients of variation were quite low for all of the different analyses on the testicles, reflecting good precision in the analyses and low animal variability.

When all of the data are considered together it seems reasonable to conclude that the 8.6 ppm of zinc in the basal diet on an as-fed basis was adequate for normal performance of the bulls to nine months of age. This level of zinc is much lower than the amounts shown to be necessary for swine (15, 17) or chicks (22). It appears that the requirement of the rat is relatively low (19). In one or more of these monogastric animals several factors have been shown to influence the zinc requirement including: source of protein (3, 16, 22); level of phytic acid (11); level of calcium, (2) at least in the presence of phytic acid (11); the addition of EDTA (1); the level of copper (14); and supplemental cadmium (18). Whether these and other factors affect the amount of zinc needed by ruminants has not been determined.

A zinc deficiency has been reported in cattle under grazing conditions (4). Samples of forage analyzed contained from 18 to 42 ppm zinc

TABLE 3

Testicle measurements and chemical composition[a] of Holstein bulls fed a low-zinc diet from 3-29 wk of age and the same diet supplemented with various levels of zinc from 30-37 wk

	Greatest diameter × length				Testicle wt fresh (castration) 37-wk		Dry matter content	Protein content Dry matter basis	Zinc content		
	29 wk		37 wk						Fresh basis	Dry matter basis	Protein basis
Group	Left	Right	Left	Right	Left	Right					
	(inches)				(g)		(%)		(ppm)		
1	5.2	5.4	7.2	6.9	127.3	127.6	12.5	76.0	10.6	84.9	111.6
2	5.7	5.5	8.6	8.3	97.0	98.6	13.7	76.3	11.6	84.7	111.1
3	4.5	4.2	6.2	6.0	98.5	99.9	13.0	77.1	11.3	86.8	112.6
4	5.5	5.9	8.4	8.2	125.1	122.9	12.9	75.6	11.0	85.7	113.3
Avg 2, 3, and 4	5.3	5.3	7.9	7.7	108.0	107.9	13.2	76.2	11.3	85.6	112.3
S_d[b]	0.85		1.02		15.0		0.48	1.9	0.44	2.7	2.4

[a] Cross section of testicle without the visceral layer and the tunica albuginea.

[b] S_d = Standard error of mean difference between Group 1 and Groups 2, 3, and 4 combined.

on a dry basis (4). The method of analyses was not given and samples may or may not have been a good reflection of the material eaten by the small percentage of the animals which developed a deficiency. However, consideration of these data in relation to the study herein reported suggests that factors other than level of zinc in the forage may have contributed to the development of their deficiency (4). Thus, it is apparent that additional studies are needed on zinc nutrition in ruminants.

ACKNOWLEDGMENTS

The authors are grateful to: Dr. J. D. Morton, Head of the Department of Veterinary Medicine and Surgery, and his associates for clinical examination of the calves; Mr. J. Stone, Jr. for technical assistance; Mrs. Rosa Nussbaumer and Mr. J. Mason for chemical analyses; Dr. W. M. Reynolds of the Chas. Pfizer Co., Terre Haute, Indiana, for vitamins and antibiotics; and Mr. J. Cooper Morcock, Jr., of the Allied Chemical Co., Atlanta, Georgia, for the urea.

REFERENCES

(1) Green, J. D., Plumlee, M. P., Smith, W. H., Parker, H. E., and Beeson, W. M. Effect of Chelating Agents on Zinc Utilization by Growing Swine. J. Animal Sci., 20: 933. 1961.

(2) Hoefer, J. A., Miller, E. R., Ullrey, D. E., Ritche, H. D., and Luecke, R. W. Interrelationships Between Calcium, Zinc, Iron and Copper in Swine Feeding. J. Animal Sci., 19: 249. 1960.

(3) Lease, J. G., Barnett, B. D., Lease, E. J., and Turk, D. E. The Biological Unavailability to the Chick of Zinc in a Sesame Meal Ration. J. Nutrition, 72: 66. 1960.

(4) Legg, S. P., and Sears, L. Zinc Sulfate Treatment of Parakeratosis in Cattle. Nature, 186: 1061. 1960.

(5) Matthews, C. A., and Fohrman, M. H. Beltsville Growth Standards for Holstein Cattle. USDA, Tech. Bull. 1099. 1954.

(6) Millar, M. J., Fisher, M. I., Elcoate, P. V., and Mawson, C. A. The Effects of Dietary Zinc Deficiency on the Reproductive System of Male Rats. Canadian J. Biochem. Physiol., 36: 557. 1958.

(7) Miller, J. K., and Miller, W. J. Development of Zinc Deficiency in Holstein Calves Fed a Purified Diet. J. Dairy Sci., 43: 1854. 1960.

(8) Miller, J. K., and Miller, W. J. Experimental Zinc Deficiency and Recovery of Calves. J. Nutrition, 76: 467. 1962.

(9) Miller, J. K., Miller, W. J., and Clifton, C. M. Calf Response to Starters of Varying Zinc Contents. J. Dairy Sci., 45: 1536. 1962.

(10) Miller, W. J., and Miller, J. K. Zinc Content of Some Feed Ingredients and Materials. J. Dairy Sci., 46: 366. 1963.

(11) Oberleas, D., Muhrer, M. E., and O'Dell, B. L. Effects of Phytic Acid on Zinc Availability and Parakeratosis in Swine. J. Animal Sci., 21: 57. 1962.

(12) Philpot, F. J., and Philpot, J. S. A Modified Colorimetric Estimation of Carbonic Anhydrase. Biochem. J., 30: 2191. 1936.

(13) Pincus, G., Miyake, T., Merrill, A. P., and Longo, P. The Bioassay of Progesterone. Endocrinology, 61: 528. 1957.

(14) Ritchie, H. D., Luecke, R. W., Baltzer, B. V., Miller, E. R., Ullrey, D. E., and Hoefer, J. A. Copper and Zinc Interrelationships in the Pig. J. Nutrition, 79: 117. 1963.

(15) Roberts, H. F., Hoekstra, W. G., and Grummer, R. H. Significance of Zinc in High-Calcium Diets for Reproducing Gilts. J. Animal Sci., 21: 1011. 1962.

(16) Smith, W. H., Plumlee, M. P., and Beeson, W. M. Zinc Requirement of the Growing Pig Fed Isolated Soybean Protein Semi-Purified Rations. J. Animal Sci., 20: 128. 1961.

(17) Smith, W. H., Plumlee, M. P., and Beeson, W. M. Effect of Source of Protein on the Zinc Requirement of the Growing Pig. J. Animal Sci., 21: 399. 1962.

(18) Supplee, W. C. Production of Zinc Deficiency in Turkey Poults by Dietary Cadmium. Poultry Sci., 40: 827. 1961.

(19) Underwood, E. J. Trace Elements in Human and Animal Nutrition. Academic Press Inc., New York. 1962.

(20) Vallee, B. L. Biochemistry, Physiology and Pathology of Zinc. Physiol. Revs., 39: No. 3. 443. 1959.

(21) Verdier, E. T., Steyn, W. J. A., and Eve, D. J. Determination of Zinc in Plants and Soils. J. Agr. Food Chem., 5: 354. 1957.

(22) Zeigler, T. R., Leach, R. M., Jr., Norris, L. C., and Scott, M. L. Zinc Requirement of the Chick: Factors Affecting Requirements. Poultry Sci., 40: 1584. 1961.

on a dry basis (4). The method of analyses was not given and samples may or may not have been a good reflection of the material eaten by the small percentage of the animals which developed a deficiency. However, consideration of these data in relation to the study herein reported suggests that factors other than level of zinc in the forage may have contributed to the development of their deficiency (4). Thus, it is apparent that additional studies are needed on zinc nutrition in ruminants.

ACKNOWLEDGMENTS

The authors are grateful to: Dr. J. D. Morton, Head of the Department of Veterinary Medicine and Surgery, and his associates for clinical examination of the calves; Mr. J. Stone, Jr. for technical assistance; Mrs. Rosa Nussbaumer and Mr. J. Mason for chemical analyses; Dr. W. M. Reynolds of the Chas. Pfizer Co., Terre Haute, Indiana, for vitamins and antibiotics; and Mr. J. Cooper Morcock, Jr., of the Allied Chemical Co., Atlanta, Georgia, for the urea.

REFERENCES

(1) Green, J. D., Plumlee, M. P., Smith, W. H., Parker, H. E., and Beeson, W. M. Effect of Chelating Agents on Zinc Utilization by Growing Swine. J. Animal Sci., 20: 933. 1961.

(2) Hoefer, J. A., Miller, E. R., Ullrey, D. E., Ritche, H. D., and Luecke, R. W. Interrelationships Between Calcium, Zinc, Iron and Copper in Swine Feeding. J. Animal Sci., 19: 249. 1960.

(3) Lease, J. G., Barnett, B. D., Lease, E. J., and Turk, D. E. The Biological Unavailability to the Chick of Zinc in a Sesame Meal Ration. J. Nutrition, 72: 66. 1960.

(4) Legg, S. P., and Sears, L. Zinc Sulfate Treatment of Parakeratosis in Cattle. Nature, 186: 1061. 1960.

(5) Matthews, C. A., and Fohrman, M. H. Beltsville Growth Standards for Holstein Cattle. USDA, Tech. Bull. 1099. 1954.

(6) Millar, M. J., Fisher, M. I., Elcoate, P. V., and Mawson, C. A. The Effects of Dietary Zinc Deficiency on the Reproductive System of Male Rats. Canadian J. Biochem. Physiol., 36: 557. 1958.

(7) Miller, J. K., and Miller, W. J. Development of Zinc Deficiency in Holstein Calves Fed a Purified Diet. J. Dairy Sci., 43: 1854. 1960.

(8) Miller, J. K., and Miller, W. J. Experimental Zinc Deficiency and Recovery of Calves. J. Nutrition, 76: 467. 1962.

(9) Miller, J. K., Miller, W. J., and Clifton, C. M. Calf Response to Starters of Varying Zinc Contents. J. Dairy Sci., 45: 1536. 1962.

(10) Miller, W. J., and Miller, J. K. Zinc Content of Some Feed Ingredients and Materials. J. Dairy Sci., 46: 366. 1963.

(11) Oberleas, D., Muhrer, M. E., and O'Dell, B. L. Effects of Phytic Acid on Zinc Availability and Parakeratosis in Swine. J. Animal Sci., 21: 57. 1962.

(12) Philpot, F. J., and Philpot, J. S. A Modified Colorimetric Estimation of Carbonic Anhydrase. Biochem. J., 30: 2191. 1936.

(13) Pincus, G., Miyake, T., Merrill, A. P., and Longo, P. The Bioassay of Progesterone. Endocrinology, 61: 528. 1957.

(14) Ritchie, H. D., Luecke, R. W., Baltzer, B. V., Miller, E. R., Ullrey, D. E., and Hoefer, J. A. Copper and Zinc Interrelationships in the Pig. J. Nutrition, 79: 117. 1963.

(15) Roberts, H. F., Hoekstra, W. G., and Grummer, R. H. Significance of Zinc in High-Calcium Diets for Reproducing Gilts. J. Animal Sci., 21: 1011. 1962.

(16) Smith, W. H., Plumlee, M. P., and Beeson, W. M. Zinc Requirement of the Growing Pig Fed Isolated Soybean Protein Semi-Purified Rations. J. Animal Sci., 20: 128. 1961.

(17) Smith, W. H., Plumlee, M. P., and Beeson, W. M. Effect of Source of Protein on the Zinc Requirement of the Growing Pig. J. Animal Sci., 21: 399. 1962.

(18) Supplee, W. C. Production of Zinc Deficiency in Turkey Poults by Dietary Cadmium. Poultry Sci., 40: 827. 1961.

(19) Underwood, E. J. Trace Elements in Human and Animal Nutrition. Academic Press Inc., New York. 1962.

(20) Vallee, B. L. Biochemistry, Physiology and Pathology of Zinc. Physiol. Revs., 39: No. 3. 443. 1959.

(21) Verdier, E. T., Steyn, W. J. A., and Eve, D. J. Determination of Zinc in Plants and Soils. J. Agr. Food Chem., 5: 354. 1957.

(22) Zeigler, T. R., Leach, R. M., Jr., Norris, L. C., and Scott, M. L. Zinc Requirement of the Chick: Factors Affecting Requirements. Poultry Sci., 40: 1584. 1961.

EFFECTS OF FLUORINE[1] ON DAIRY CATTLE. IV. MILK PRODUCTION[2]

G. E. STODDARD, G. Q. BATEMAN, L. E. HARRIS, J. L. SHUPE,[3] AND D. A. GREENWOOD

Departments of Dairy Industry, Animal Husbandry, Veterinary Science, and Biochemistry
Utah State University, Logan

SUMMARY

Thirty-two Holstein females were maintained from three to four months of age until 7.5 yr of age on diets containing four levels of fluorine, two levels of concentrate, and two levels of a calcium-phosphorus mineral.

Statistically, only the concentrate level treatment affected milk production and then only in the first lactation. Combined lactation, histological, pathological, and other data, however, indicate a reduced milk production on 109 ppm F treatment in the third and fourth lactation and possibly in the second. The 55 ppm treatment appeared to adversely affect milk production in the fourth lactation. Cows on the higher concentrate treatment tended toward higher milk production than did those on the lower level. Mineral treatment did not affect lactation when missing values were not calculated.

Efficiency of lactation was not affected by F intake, although higher concentrate and 1% mineral treatments induced more efficient production than the lower concentrate and 3% mineral treatments.

All milk residues of F were within safe levels for consumption, although residues increased with increased dietary F.

Based on this study, the tolerance of F for lactation in dairy cows over a 7.5-yr period is less than 55 ppm on a dry hay basis or less than 49 ppm on a total dry diet basis.

Ingestion of excessive amounts of fluoride over a sufficient period of time has been shown to have an adverse effect on animals (1, 3-8, 10, 11-23). Some of these effects such as tooth lesions and periosteal hyperostosis are direct; others, such as reduced milk production, may result from such a factor as reduced feed consumption and would be classed as indirect. Both types of effects have been observed in studies with dairy cows (10-13, 15-23).

Tooth structure is affected only during the formative period of the teeth. If effects are sufficiently severe to interfere with mastication, a reduction in feed consumption may follow which, in turn, may cause a drop in milk production. The age of the animal when the fluoride is ingested is, therefore, an important factor. Most studies on milk production have involved cows placed on elevated fluorine levels at milking age, after many of the permanent teeth have already formed and some have erupted. Dental lesions and any related influences are less severe for such animals than for those exposed to similar intakes during their entire growth period.

Since dairy animals exposed to industrial fluorides are usually exposed at early ages as well as during their productive life, a study was conducted to observe the effects of such exposure on a group of Holstein females.

In a previous publication (19) data were reported on feed consumption and growth. This report is concerned with the effects on milk production.

EXPERIMENTAL PROCEDURE

Details of the experimental design are given in a previous report (19). The procedure is only briefly described here.

Received for publication February 28, 1963.

---

[1] Fluorine, extremely active, occurs naturally only as the various fluorides. In this report fluorine and fluoride are used interchangeably to designate fluorine in its various combinations. Added fluorine was in the form of sodium fluoride.

[2] Published with the approval of the Director of the Utah Agricultural Experiment Station as Journal paper No. 319. This research was supported in part by a grant-in-aid from Columbia-Geneva Division, United States Steel Corporation, Provo, Utah.

[3] Current address: Research Veterinarian, ADP-ARS, Logan, Utah.

Thirty-two Holstein dairy females, with an average weight of 105 kg (three to four months of age), were divided into two replications based on body weight and were assigned at random within replication to 16 treatments, as shown in Table 1.

The trial started on April 16, 1952, and continued until July 1, 1959. Animals were sacrificed in a random manner from July 2, 1959, to September 3, 1959. Other than imposed experimental treatments, all animals had equal opportunity to freshen regularly and to produce for an equal period of time. Variations are expressions of individual variations and experimental treatment effects. Analyses of variance and covariance were employed to aid in detecting treatment effects.

Fluorine levels were planned to compare effects of intake of normal hay and hay containing fluorine residues of 25, 50, and 100 ppm on a dry basis. Average levels on a hay basis as determined by chemical analyses for the four treatments were 10, 28, 55, and 109 ppm, respectively, and these values will be used in referring to the four treatments.

A Ca-P mineral was fed as 1 and 3% of the concentrate mix, to determine the influence of such minerals on fluorosis symptoms in dairy females. Defluorinated phosphate was fed from April 16, 1952 (start of the trial) until March 30, 1954, and steamed bone meal from March 31, 1954, until the end of the experiment.

Two levels of the concentrate mix were fed. During nonlactating periods one group (lower) was fed 2 lb daily and the other (higher) 4 lb daily (19). During lactation, the higher group received 1 lb of grain daily for each pound of milk fat produced weekly. The lower group received 0.75 lb of grain daily for each pound of milk fat produced weekly. All animals were fed individually.

Milk production was recorded, starting on the third day following parturition, and cows were milked until about 6 wk before the next calculated calving date. Cows were dried off when daily milk production dropped to less than 5 lb, however, even though this occurred before the date they were due dry.

Milk samples were taken for fluorine analysis during each lactation, 60 to 90 days after parturition. Fluorine was determined by a perchloric acid distillation following the procedure of Willard and Winter (24) and concentrating the fluorine on an ion exchange resin (9). Milk was weighed after each milking and analyzed for milk fat (2) twice monthly. Milk and fat were converted to a 4% fat-corrected milk (FCM) standard according to the formula FCM = (0.4) (lb milk) + (15) (lb milk fat). A factor of efficiency was calculated as:

$$\frac{\text{g FCM produced}}{\text{100 kcal digestible energy consumed}}$$

RESULTS AND DISCUSSION

Summaries of lactations are presented in Tables 2 and 3. Production was equated to 4% fat-corrected-milk (FCM) for total lactation periods and average daily production. For the first four lactations, missing values were

TABLE 1

Experimental treatments

Concentrate mix	Ca-P mineral in concentrate mix	Fluorine level (ppm)			
		Based on avg hay plus F added			
		10	28	55	109
		Based on dry matter			
		12	27	49	93
	(%)	(No. of animals)			
Lower	1	2	2	2	2
	3	2[a]	2	2	2[b]
	1	2	2	2	2[c]
Higher	3	2	2	2	2[d]

[a] Forty-five days after trial started one heifer developed actinobacillosis and was replaced with another heifer.

[b] One heifer was sacrificed on January 10, 1956, after about 3 years 9 months on the experiment. She was severely lame in both fore limbs and the right hind limb and was unable to walk.

[c] One heifer was sacrificed on March 3, 1954, after almost 1 year 11 months on the experiment because of a severe nonresponsive case of edometritis.

[d] One heifer was sacrificed on August 30, 1956, after about 4 years 4 months on the experiment. She developed a large spinal abscess between the sixth and seventh thoracic vertebrae, that resulted in paralysis of the hind limbs.

TABLE 2

Lactation summaries (FCM) of first four total lactations and total of all lactations of Holstein females fed four levels of fluorine from three to four months of age to about 7.5 yr of age

Lactation	Fluorine level (ppm) based on hay intake											
	10			28			55			109		
		Milk			Milk			Milk			Milk	
		Total	Daily		Total	Daily		Total	Daily		Total	Daily
(no.)	(days)	(kg)	(kg)	(days)	(kg)	(kg)	(days)	(kg)	(kg)	(days)	(kg)	(kg)
1	337	4,200	12.4	302	3,463	11.3	323	3,752	11.6	320*	3,796*	11.8*
2	353	4,866	13.8	307	4,049	13.2	333	3,678	11.1	334	4,187*	12.6*
3	352	5,191	14.6	304	3,887	12.6	321	4,604	14.0	[a]	3,664*	11.8*
4	330	4,793	14.5	327	4,706	14.4	312*	3,607*	13.0*	[a]	3,808*	12.2*
Avg	343	4,762	13.8	310	4,026	12.9	322*	3,910*	12.5*	[a]	3,864*	12.2*
Total all lactations[b]	1,657	23,595	14.2 14.2	1,611	2,190	13.6 13.6	1,490	19,335	13.0	1,096	14,143	11.1 12.9

* Missing values included in calculation.

[a] Missing values not calculated.

[b] Includes lactations beyond fourth and does not include any calculated missing values. Totals and averages include only seven cows for 109 ppm treatment. Cow 21 with obstruction in all four quarters not considered in treatment effect on this basis. The daily averages are averages of individual cow averages except those on the bottom line of the table, which were obtained by dividing total production by total days in milk.

TABLE 3

Lactation summaries (FCM) of first four total lactations and total of all lactations of Holstein females fed two levels of a concentrate mix and two levels of a Ca-P mineral from three to four months of age to about 7.5 yr of age

Lactation	Concentrate level						Ca-P mineral level					
	Lower			Higher			1%			3%		
		Milk			Milk			Milk			Milk	
		Total	Daily		Total	Daily		Total	Daily		Total	Daily
(no.)	(days)	(kg)	(kg)	(days)	(kg)	(kg)	(days)	(kg)	(kg)	(days)	(kg)	(kg)
1	312	3,483	11.1	328*	4,122*	12.4*	312*	3,890*	12.1*	329	3,716	11.5*
2	326	3,942	12.2	341*	4,448*	13.1*	324*	4,437*	13.7*	344	3,952	11.7*
3	[a]	3,821*	12.3*	[a]	4,841*	14.2*	[a]	4,600*	13.8*	[a]	4,073*	12.7*
4	[a]	4,099*	12.8*	[a]	4,358*	14.5*	[a]	4,086*	13.4*	[a]	4,371*	14.0*
Avg	319*	3,839*	12.1*	334*	4,442*	13.5*	318*	4,235*	13.2*	336*	4,028*	12.5*
Total all lactations[b]	1,515	19,562	12.3 12.9	1,438	20,293	13.6 14.1	1,435	19,994	13.0 13.9	1,512	19,936	13.0 13.2

* Missing values included in calculation.

[a] Missing values not calculated.

[b] Includes lactations beyond fourth and does not include any calculated missing values. Totals and averages include only 15 cows for lower concentrate and 1% mineral treatments. Cow 21 with obstruction in all four quarters not considered in treatment effect on this basis. The daily averages are averages of indivdiual cow averages, except those on the bottom line of the table, which were obtained by dividing total production by total days.

calculated for cows that were sacrificed or did not lactate. Cow no. 21 had anatomical obstructions in all four teat canals and attempts to open them surgically were not successful. Cows 11, 12, and 28 were sacrificed, as indicated at the bottom of Table 1.

Since there was no evidence of interference by fluoride or mineral treatments during the first lactation, the subsequent lactations were analyzed by covariance, taking into account first-lactation variance. Analysis of variance of the first lactation indicated a significantly greater ($p < .05$) production by the higher than the lower concentrate group.

Before certain lactation data were analyzed statistically, missing values were calculated for the cows that were sacrificed, or did not lactate. When the data were calculated in this way, the treatments did not significantly affect milk production. Considering the individual variation in lactation that would be expected by a group of dairy cows selected as these were as young calves, and with the limited numbers on each treatment, statistical interpretation of treatment effect on lactation has only limited value. Since the three animals that were sacrificed, and the one with anatomical obstruction in the teat canals, were all on the highest fluorine level, the actual total production of that group was accordingly the lowest of any of the treatment groups (Table 2). The influence of fluoride treatment on these animals is discussed by Shupe et al. (18). Since Animals 11, 12, and 28 did not respond to corrective medical treatment as expected, the ingested fluorides may have had an adverse influence on them. Total FCM production for all lactations on each treatment is shown at the bottom of Tables 2 and 3. Since all animals had equal opportunity to produce (excluding Cow 21), except for genetics and experimental treatment, fluorine had an adverse effect on total milk production at the two higher levels of fluorine intake (Figure 1). The small differ-

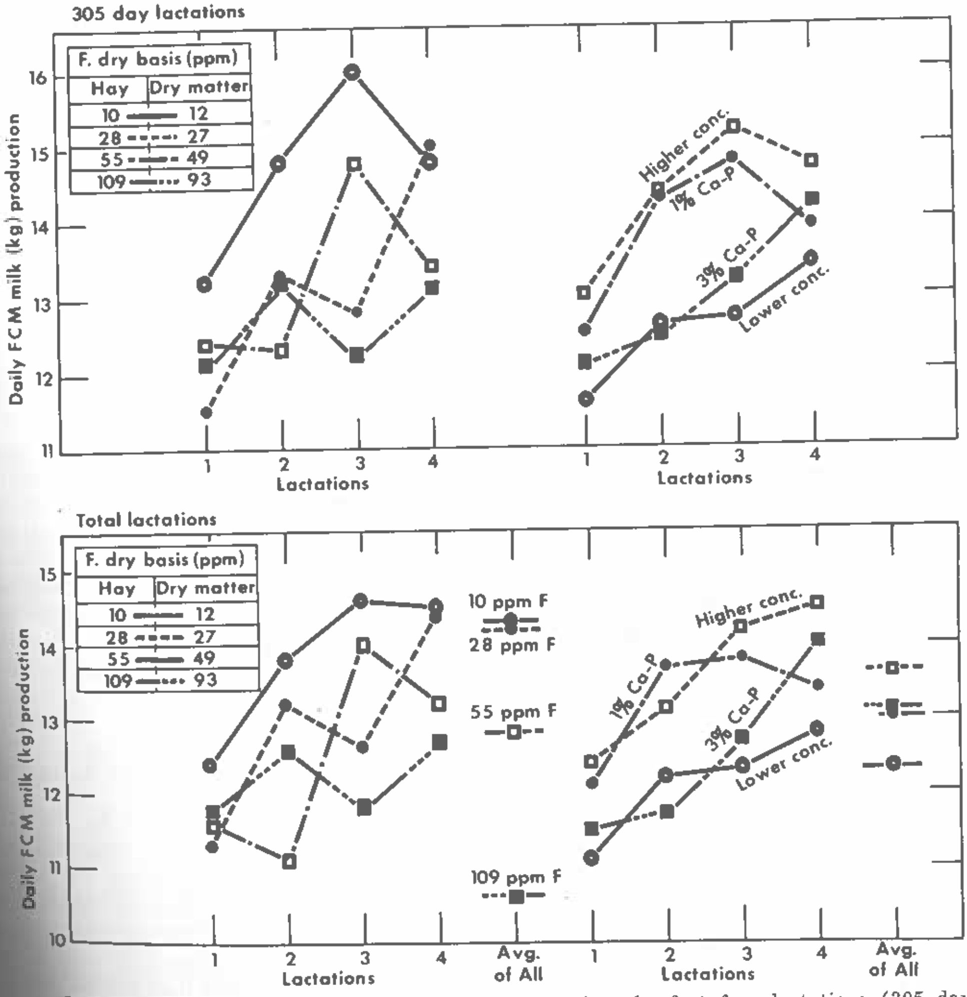


FIG. 1. Average* daily milk (FCM) produced during the first four lactations (305 day and total) of Holstein females fed four levels of fluorine, two levels of concentrate mix, and two levels of a Ca-P mineral from three to four months of age to about 7.5 yr of age.

* Averages for individual lactations include calculated missing values. Averages for all lactations include no missing values.

ence in milk production between animals on the two lower levels of fluorine is considered within normal biological variation. Cows on the 28, 55, and 109 ppm F treatments produced 93, 82, and 60% as much FCM as the 10 ppm F animals.

Total days in milk were reduced for animals on the 55 and 109 ppm F treatment. The reduction was associated with reduced number of lactations and not with shortened lactation periods. Average daily milk (FCM) production is shown in Table 2. All values except those in the last line are averages of individual cow averages and give equal weight to each cow. The last line of the table, however, includes averages calculated from total days in milk and total production for the entire experiment. These latter values give greater emphasis to those cows with more production and less to those with less production. By both methods of calculation, average daily milk production decreased with increased fluorine intake. The difference between the 10 and 28 ppm F animals is considered to be genetic, since the difference existed even in the first lactation when data showed no lactational effects on animals receiving any of the fluoride treatments.

Neither concentrate level nor mineral level significantly influenced total milk production for the entire trial. There was, however, a higher daily milk production by cows on the higher grain intake (Figure 1). When missing values were calculated, there was a tendency for cows on 1% mineral to produce more than those on 3% mineral during the first three lactations.

Milk production effects of fluorine ingestion at the levels fed in this experiment appear to be secondary rather than primary. Also, within the levels of fluorine intake included in this study, several characteristic clinical symptoms and elevated bone fluorine content preceded milk production decline. Shupe et al. (18) reported that animals on the 55 ppm F diet showed palpable and discernable bone lesions after 3.5-4 yr on experiment and lameness after 4.5 yr. On 109 ppm F, similar symptoms appeared after 1.5-2 yr and 2.5 yr, respectively. Feed consumption and body weight declined in the 109 ppm F animals after about 2.5-3 yr on trial, whereas the animals on 55 ppm F did not show such a decline during the trial (19). Milk production effects followed these other manifestations of elevated fluoride intake. In the third lactation, cows on the 109 ppm F treatment showed a decline in milk production (Figure 1). There may have been some decline in the second lactation, although the magnitude of the decline, if it actually exists, could not be determined with the available data.

The 55 ppm fluorine cows showed a decline in average FCM production in the second lactation, but this was a result of a decline for two cows. One of these had a large encapsulated abscess in the right paralumbar fossa region and was sick for a considerable part of her early lactation. Production declined and did not increase appreciably even after successful removal of the abscess. In their third lactations these two cows were two of the highest milk producers on the trial, indicating that the earlier decline was not a treatment effect. During the fourth lactation, animals on this treatment appeared to be adversely affected. The production decline was preceded by clinical fluorosis symptoms, a factor supporting the treatment effect on production decline (18).

Results of this trial indicate that the tolerance level for fluorine for lifetime (7.5 yr) milk production is less than 55 ppm on a dry hay basis or 49 ppm on a total dry diet basis. No lactation effects were observed in animals receiving 27-28 ppm F, indicating that the tolerance for milk production is somewhere between 27 and 49 ppm F on a total dry diet basis or 28 and 55 ppm F on a dry hay basis. Certain clinical and pathological changes preceded lactation decline in the cows fed elevated levels of fluoride (18).

Effects on lactation were similar to those noted by Suttie et al. (21), who started heifers on experiment at 2 yr of age. Some of their animals receiving 50 ppm of added fluorine showed a lactational affect in the third lactation, whereas those on the lower intake showed no such effect. Lactation effects were associated with a reduced feed intake. Newell and Schmidt (10) indicated that 2.0 to 2.5 mg F per kilogram body weight is the level at which toxicosis may be expected to occur after periods of prolonged ingestion by dairy cows, although at these levels they noted no adverse effects on milk production for five lactations starting with 2-yr-old heifers.

There appears to have been no fluoride treatment effect on the grams of FCM produced per 100 kcal of digestible energy (Figure 2). A comparison of efficiency levels within lactations did not show any fluorine treatment effect. The 1% Ca-P mineral treatment was consistently more efficient than the 3%, and the higher concentrate treatment was more efficient than the lower concentrate treatment.

Fluorine contents of milk during third, fourth, and fifth lactations are shown in Table 4. Resi-

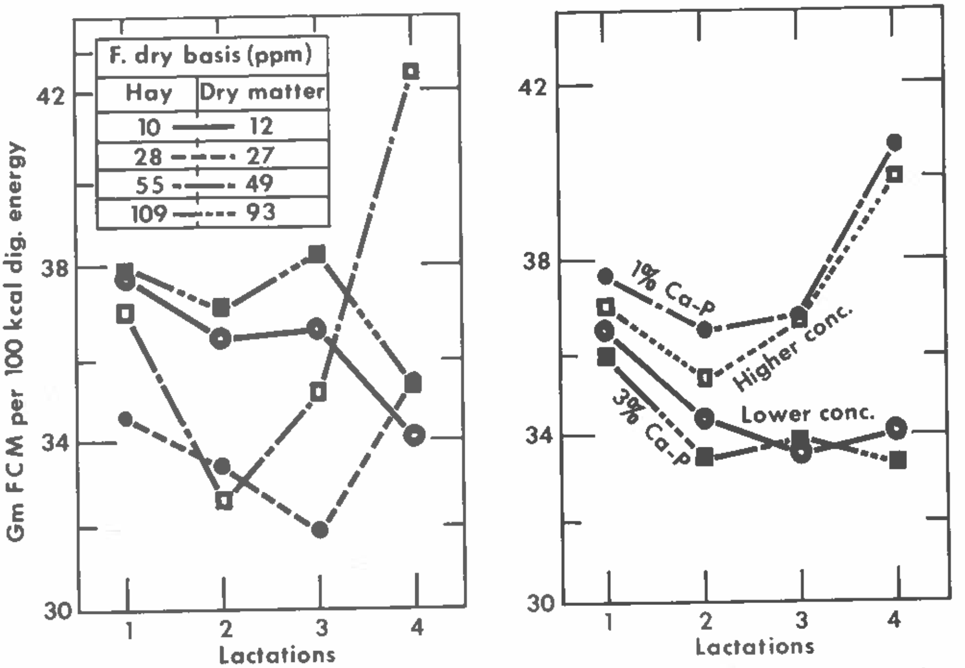


Fig. 2. Grams of FCM produced per 100 kcal of digestible energy during first four lactations of Holstein females fed four levels of fluorine, two levels of concentrate mix, and two levels of a Ca-P mineral from three to four months of age to about 7.5 yr of age.

TABLE 4

Fluorine (ppm) in whole milk of Holstein females fed four levels of fluorine from three to four months of age to about 7.5 yr of age

Fluorine level (ppm)	Fluorine (ppm) in milk[a]		
	Lactations		
	3	4	5
10	0.036	0.096	0.060
28	0.049	0.099	0.098
55	0.078	0.127	0.137
109	0.096	0.172	0.198

[a] Sample taken during period 60-90 days after parturition.

dues of fluorine are positively correlated with fluoride treatment, but all values are within safe levels for human consumption. Physiologically, the results show the mammary gland to be a minor means of excretion. From a practical point of view, however, within the range of fluorine intake of this experiment the fluorine content of milk would not be so high as to constitute a health hazard.

ACKNOWLEDGMENT

The authors thank John E. Butcher, A. G. McKinnon, C. H. Mickelsen, M. L. Miner, H. M. Nielsen, A. E. Olson, Robert J. Raleigh, and Joseph C. Street for their help in carrying out this research.

REFERENCES

(1) Agate, J. N., Bell, G. H., Bodie, G. F., Bowler, R. G., Bucknell, M., Cheeseman, E. A., Douglas, L. S., Druett, H. A., Gerrad, J., Hunter, D., Perry, K. M. A., Richardson, J. D., and deV. Weir, J. B. Industrial Fluorosis. Great Britain Med. Research Council, Memo. 22. 1949.

(2) Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. 7th ed. Compiled by the Committee on Editing Methods of Analysis. Washington, D. C. 1950.

(3) De Eds, F. Chronic Fluorine Intoxication. Ann. Rev. Med., 12: 1. 1933.

(4) Gettler, A. O., and Ellerbrook, L. Toxicology of Fluorides. Am. J. Med. Sci., 197: 625. 1939.

(5) Greenwood, D. A. Fluoride Intoxication. Physiol. Revs., 20: 528. 1940.

(6) Huffman, C. F., and Reed, O. E. Results of a Long-Time Mineral Feeding Experiment with Dairy Cattle. Michigan Agr. Expt. Sta., Bull. 129. 1930.

(7) McClure, F. J. A Review of Fluorine and Its Physiological Effects. Physiol. Revs., 13: 277. 1933.

(8) Mitchell, H. H., and Edman, M. The Fluorine Problem in Livestock Feeding. Nutr. Abstr. Revs., 21: 787. 1952.

(9) Neilsen, H. M. Determination of Microgram Quantities of Fluoride. Anal. Chem., 30: 1009. 1958.

(10) Newell, G. W., and Schmidt, H. J. The Effects of Feeding Fluorine as Sodium Fluoride to Dairy Cattle—A Six Year Study. Am. J. Vet. Research, 19(71): 363. 1958.

(11) Phillips, P. H., Greenwood, D. A., Hobbs, D. S., and Huffman, C. F. The Fluorosis Problem in Livestock Production. National Academy of Sciences—National Research Council, Publ. 824. 1960.

(12) Phillips, P. H., Hart, E. B., and Bohstedt, G. Chronic Toxicosis in Dairy Cows Due to Ingestion of Fluorine. Wisconsin Agr. Expt. Sta., Research Bull. 123. 1934.

(13) Reed, O. E., and Huffman, C. F. The Results of a Five Year Mineral Feeding Investigation with Dairy Cattle. Michigan Agr. Expt. Sta., Tech. Bull. 105. 1934.

(14) Roholm, K. Fluorine Intoxication. A Clinical-Hygienic Study. H. K. Lewis and Company, London, England. 1937.

(15) Schmidt, H. J., Newell, G. W., and Rand, W. E. The Controlled Feeding of Fluorine as Sodium Fluoride to Dairy Cattle. Am. J. Vet. Research, 15: 232. 1954.

(16) Schmidt, H. J., and Rand, W. E. A Critical Study of the Literature on Fluoride Toxicology with Respect to Cattle Damage. Am. J. Vet. Research, 13: 38. 1952.

(17) Shupe, J. L., Miner, M. L., Binns, W., Greenwood, D. A., Neilsen, H. M., Stoddard, G. E., Bateman, G. Q., and Harris, L. E. Pathology of Chronic Bovine Fluorosis. Proc. Am. Vet. Med. Assoc. 92nd Ann. Mtg. Minn. August 15-18, 1955. pp. 195-201. 1955.

(18) Shupe, J. L., Miner, M. L., Harris, L. E., Greenwood, D. A., and Stoddard, G. E. The Effect of Fluorine on Dairy Cattle. II. Clinical and Pathological Effects. J. Vet. Research. (In press.) 1963.

(19) Stoddard, G. E., Harris, L. E., Bateman, G. Q., Shupe, J. L., and Greenwood, D. A. Effects of Feeding Various Levels of Fluorine, Calcium, Phosphorus, and Concentrate Mix to Dairy Females from Weaning to Maturity. I. Growth and Feed Consumption. Unpublished results. 1963.

(20) Suttie, J. W., Gesteland, R., and Phillips, P. H. Effects of Dietary Sodium Fluoride on Dairy Cows. VI. In Young Heifers. J. Dairy Sci., 44: 2250. 1961.

(21) Suttie, J. W., Miller, R. F., and Phillips, P. H. Studies of the Effects of Dietary NaF on Dairy Cows. II. The Effects on Milk Production. J. Dairy Sci., 40: 1485. 1957.

(22) Suttie, J. W., Miller, R. F., and Phillips, P. H. Studies of the Effects of Dietary NaF on Dairy Cows. I. The Physiological Effects and the Developmental Symptoms of Fluorosis. J. Nutrition, 63: 211. 1957.

(23) Suttie, J. W., and Phillips, P. H. Studies of the Effects of Dietary Sodium Fluoride on Dairy Cows. V. A Three-Year Study on Mature Animals. J. Dairy Sci., 42: 1063. 1959.

(24) Willard, H. H., and Winter, O. B. Volumetric Method for Determination of Fluorine. Ind. Eng. Chem., Anal. Ed., 5: 7. 1953.

ENSILING CHARACTERISTICS OF COASTAL BERMUDAGRASS HARVESTED AT THE PREHEAD AND FULL-HEAD STAGES OF GROWTH[1]

W. J. MILLER, C. M. CLIFTON, AND N. W. CAMERON
Dairy Department, University of Georgia, Athens

SUMMARY

In a study of the influence of growth stage at harvest on changes occurring during ensiling, four 8- by 24-ft. tower silos were rapidly filled with Coastal Bermudagrass *(Cynodon dactylon)* at the (a) prehead and (b) full-head stages. Ground shelled corn was added at the rate of 100 lb per ton of forage. The younger forage contained more protein and less NFE, but the levels of crude fiber and dry matter were not materially affected by harvesting stage. Level of ammoniacal N and pH were approximately the same for silages made from the prehead and full-head Coastal Bermudagrass. The percentages of organic acids were quite low for all silages with prehead silage containing somewhat more lactic acid. On a dry matter basis total organic acids were 4.2 and 4.1% for the prehead and full-head silages. Nutrient losses by both fermentation and spoilage, as determined by the input-output method, were larger for the more mature material. Temperatures developed during ensiling were approximately the same for all four silages, with average peak values of 104 and 105 F in silos with prehead and full-head material.

Because of its excellent agronomic properties, Coastal Bermudagrass has become a very important forage crop throughout the southern United States. Information regarding suitable ways to utilize it in dairy feeding programs has lagged behind agronomic research. Few studies of Coastal Bermudagrass silage are found in the literature. Consequently, little is known about the changes which occur during the ensiling of this forage or the factors which affect these changes. With some forages harvesting stage has important effects on the chemical changes, nutrient losses, and temperatures occurring during ensiling (13, 14).

The purpose of this experiment was to study the chemical changes, nutrient losses, and temperature elevations which occur when Coastal Bermudagrass is ensiled at the prehead and the full-head stages of development.

EXPERIMENTAL PROCEDURE

Two 8- by 24-ft tower silos were filled with Coastal Bermudagrass in the prehead stage, just prior to head emergence, and two with comparable forage in the full-head stage.

The Coastal Bermudagrass was harvested with a forage harvester,[2] using a ¼-in. theoretical cut. Ground shelled corn at the rate of 100 lb per ton of forage was added to each silo. To improve silage quality and minimize fermentation losses the silos were filled rapidly (11), with none of the material exposed for more than 6 hr before sealing with 4-mil black polyethylene covers weighted with 3 in. of sawdust.

The forage was grown on Cecil sandy clay loam and had been fertilized with 600 lb of 5-10-15 ($N—P_2O_5-K_2O$) on April 11, 1961, and 66 lb of N as NH_4NO_3 per acre immediately following the harvests which preceded each of those used experimentally. Forage for the first comparison was from the second cutting of the season, whereas that of the second comparison was from the third cutting. Harvest dates for the prehead and full-head forages in the two cuttings were July 10, July 24, August 9, and August 21, 1961, respectively. Due to drought and cool weather a longer growing period was required for the forages to reach the desired stages in the second cutting than in the third. These values were 42 and 30 days for the prehead stage and 56 and 42 days for the full-head stage, in the two cuttings, respectively. Average extended plant heights of the prehead and

Received for publication February 18, 1963.

[1] Journal Paper No. 280 of the College Experiment Station, University of Georgia, College of Agriculture Experiment Stations.

[2] New Holland Model No. 818.

the full-head Coastal Bermudagrass plants were 15 and 22 in.

Yields were obtained by weighing the forage removed, determining dry matter, and measuring the land area. The silages from Cuttings 2 and 3 were stored for seven and five months before being weighed and sampled. The silage from each silo was divided into approximately 40 portions of 300-500 lb each, which were thoroughly mixed and sampled. The dry matter content of the forage was determined by oven drying and that of the silage by both toluene distillation and oven-drying methods (17). Ammoniacal N, crude protein, organic acids (18), pH, and dry matter were determined on fresh or frozen samples. Analyses for the other constituents were performed on dried samples. Other details of sampling, chemical analyses, and temperature measurement procedures have been described previously (11-14) and are in conformity with recommended procedures (17).

RESULTS AND DISCUSSION

Chemical composition data of the forages, the forages plus corn, and the silages are presented in Table 1. In the second cutting, the dry matter contents of the prehead and full-head Coastal Bermudagrass were essentially the same. This appeared to be owing to the more humid weather at the time of the later harvest, and slow drying-out caused by the thick, rank growth and lodging. Coastal Bermudagrass is normally high in dry matter relative to many forages and as it matures the moisture content changes relatively less than does that of more succulent forages, such as many of the cereals. The silage contained

TABLE 1

Chemical composition of the Coastal Bermudagrass forages, ensiled mixtures, and silages

	Prehead			Full-head		
	2nd cutting	3rd cutting	Avg	2nd cutting	3rd cutting	Avg
Dry matter *(% of total wt)*						
Forage[b]	29.9	28.2	29.1	28.5	31.5	30.0
Forage + corn	32.7	31.1	31.9	31.5	34.2	32.9
Silage (Toluene d.m.)[a]	32.5	29.0	30.8	30.7	32.6	31.7
Silage (Oven d.m.)[b]	30.6	28.2	29.4	27.6	29.8	28.7
Crude protein *(% of dry matter)*						
Forage	14.8	15.2	15.0**	12.3	11.0	11.7**
Forage + corn	14.0	14.3	14.2	11.8	10.8	11.3
Silage	13.3	12.4	12.9**	10.9	10.1	10.5**
Ether extract *(% of dry matter)*						
Forage	2.2	1.9	2.1**	1.8	1.6	1.7**
Forage + corn	2.4	2.2	2.3	2.1	1.9	2.0
Silage	3.7	3.2	3.5**	2.5	2.8	2.7**
Crude fiber *(% of dry matter)*						
Forage	32.4	33.5	33.0	33.8	32.9	33.4
Forage + corn	28.5	29.2	28.9	29.4	29.1	29.3
Silage	30.5	31.2	30.9	31.6	29.7	30.7
Ash *(% of dry matter)*						
Forage	5.1	5.6	5.4	5.1	5.0	5.1
Forage + corn	4.6	5.0	4.8	4.6	4.5	4.6
Silage	5.1	6.0	5.6	5.1	5.2	5.2
NFE *(% of dry matter)*						
Forage	45.5	43.8	44.7**	47.0	49.5	48.3**
Forage + corn	50.5	49.3	49.9	52.1	53.7	52.9
Silage	47.4	47.2	47.3**	49.9	52.2	51.1**
Ammoniacal N of silage						
As N *(% of dry matter)*	0.54	0.57	0.6	0.64	0.35	0.5
As % of total N	25.4	28.8	27.1	36.8	21.6	29.2
pH of silage	5.0	5.3	5.2	5.1	5.4	5.3

** Averages significantly different at the 1% level.

[a] Dry matter determined by toluene distillation.

[b] Dry matter determined by oven drying.

slightly less dry matter than did the material ensiled. There was no seepage and the silos had a roof. Thus, any real increase in moisture content is attributable to water produced by respiration and fermentation.

Dry matter content of the silages as determined by the oven-drying procedure was appreciably lower ($P < 0.01$) than the values obtained by toluene distillation. This difference is due to the volatile materials lost in drying. However, the magnitude of the difference between these two measures does not appear to be closely related to silage quality (3). With the prehead Coastal Bermudagrass the difference between dry matter, determined by the oven-drying and toluene distillation, was only one-half as large as with the forage harvested at the full-head stage.

The prehead forage contained 3.3 percentage points or 28% more ($P < 0.01$) crude protein on a dry basis than did the older material. This change with age has been observed previously with Coastal Bermudagrass (16), as well as with other forages. During the ensiling and storage period the protein content declined in all the silos. Other than protein, NFE and ether extract were the only chemical fractions of the forage materially affected by stage of maturity at harvest ($P < 0.01$).

The crude fiber content was about the same for the Coastal Bermudagrass harvested in the prehead and full-head stages, for both the second and third cuttings. Failure of this component to increase appreciably as Coastal Bermudagrass grows older has been observed previously (5, 10). In a study, in which Coastal Bermudagrass was harvested for pellets at 3, 5, and 7-wk cycles throughout the season, the crude fiber content only increased slightly with age with average values of 28.0, 30.2, and 30.9%, respectively (10). However, milk production of cows fed the pellets declined as the age at harvest increased (10). Digestibility also decreased as the Coastal Bermudagrass matured, with average organic matter digestibilities of 66.0, 60.7, and 56.8% for the forages harvested at 3-, 5-, and 7-wk frequencies (9). Thus, it appears that under some conditions the percentage of crude fiber may not be a very reliable measure of nutritive value in Coastal Bermudagrass.

The ammoniacal N content of the silages represented a rather sizable proportion of the total N (28%), but was not consistently or materially affected by stage of maturity at filling (Table 1). A high level of ammoniacal N is generally associated with poor silage preservation and reduced dry matter consumption (1, 4, 15). By some standards the amount in these silos would not be considered indicative of good-quality silage (1, 15).

Silage made from the prehead Coastal Bermudagrass contained more lactic acid ($P < 0.05$) but no more total organic acids than did silages made from the full-head material (Table 2). The level of propionic acid was about the same for silages from the two stages of grass. The slightly lower levels of butyric, acetic, and formic acids in silages made from the prehead Coastal Bermudagrass were not significant ($P < 0.20$). All of the silages contained extremely small amounts of volatile organic acids (Table 2), relative to other materials which have been ensiled under comparable conditions (11). In this respect, as with several others, Coastal Bermudagrass appears to have rather unusual ensiling characteristics. It has been shown previously that alfalfa haylage contains a lower level of organic acids than does silage (4). While direct-cut Coastal Bermudagrass has a considerably lower dry matter content than that typical of haylage, the silage has some of the appearance of haylage. Coastal Bermudagrass produces stiff stems, difficult to

TABLE 2

Organic acid composition of the silages

	Prehead			Full-head		
	2nd cutting	3rd cutting	Avg	2nd cutting	3rd cutting	Avg
	(% of dry matter)					
Butyric Acid	0.6	0.8	0.7	1.2	0.7	1.0
Propionic Acid	0.7	0.5	0.6	1.1	0.3	0.7
Acetic Acid	0.8	1.1	1.0	1.5	0.9	1.2
Formic Acid	0.1	0.1	0.1	0.2	0.1	0.2
Lactic Acid	1.8	1.9	1.9[a]	0.6	1.6	1.1[a]
Total	4.0	4.4	4.2	4.6	3.6	4.1

[a] Averages significantly different at the 5% level.

compress. An average of only 19.2 lb of Coastal Bermudagrass and corn, or 6.2 lb of dry matter per cubic foot, was put in the silo at the time of filling.

Losses during ensiling and the amounts of nutrients recovered in the silages are presented in Table 3. Values of dry matter were calculated on the basis of total dry matter ensiled and with the assumption that all of the losses should be charged to the forage. The latter method was used for presenting the balance data for the other nutrients. It is to be expected, of course, that the ensiling losses will originate both from forage and from the corn nutrients ensiled. However, as all of the corn would have been available for feeding if it had not been put in the silo, it seems more reasonable to charge all of the losses to the preservation of the forage. Sufficient information is presented to allow one to make calculations on the basis of total nutrients ensiled for each of the components.

In spite of careful sealing, some spoilage was observed throughout the silage. Silos have been filled previously under approximately the same conditions with Sudangrass; oats; oats, ryegrass, and crimson clover; and rye, ryegrass, and crimson clover, with little or no spoilage (11-14). Thus, the Coastal Bermudagrass appears to be decidedly more difficult to preserve than these forages. Whereas the Coastal Bermudagrass is higher in dry matter than most of these materials, two silos were filled previously which contained a comparable level of dry matter, and little or no spoilage was experienced (12, 13).

The average losses by both spoilage and fermentation were appreciably higher for the

TABLE 3

Ensiling losses and nutrient recovery as measured by in-put out-put data

	Prehead			Full-head		
	2nd cutting	3rd cutting	Avg	2nd cutting	3rd cutting	Avg
Total *(% of total wt)*						
Spoiled silage	5.7	7.2	6.5*	12.5	12.4	12.5*
Invisible loss	0.7	1.9	1.3**	7.7	7.7	7.7**
Good silage	93.6	90.9	92.3*	79.7	80.0	79.9*
Dry matter *(% of total d.m.)*[a]						
Spoiled silage	4.6	6.7	5.7	7.1	13.5	10.3
Invisible loss	2.3	8.4	5.4[e]	15.1	10.3	12.7[e]
Good silage	93.1	84.9	89.0*	77.8	76.3	77.1*
Dry matter *(% of forage d.m.)*[b]						
Spoiled silage	5.2	7.7	6.5	8.2	15.3	11.8
Invisible loss	2.6	9.7	6.2	17.6	11.7	14.7
Good silage	92.1	82.6	87.4[e]	74.2	73.0	73.6[e]
Good silage[d]	(85.9)	(79.9)	(82.9)*	(65.2)	(65.4)	(65.3)*
Crude protein as silage *(% of forage protein)*	87.1	71.3	79.2	68.9	68.2	68.6
Crude fiber as silage *(% of forage crude fiber)*	99.6	90.6	95.1[e]	83.4	77.8	80.6[e]
NFE as silage[c] *(% of forage NFE)*	84.0	75.7	79.9[e]	67.2	68.0	67.6[e]
Ether extract as silage *(% of forage ether extract)*	153.6	135.6	144.6[e]	87.6	116.7	102.2[e]
Ash as silage *(% of forage ash)*	104.1	101.3	102.7**	85.7	87.1	86.4**

* Averages significantly different at the 5% level.

** Averages significantly different at the 1% level.

[a] d.m.—dry matter.

[b] Assumes all of corn nutrients recovered as good silage.

[c] NFE = Nitrogen-free extract.

[d] Data in ( ) based on oven dry matter. All others based on dry matter determined by toluene distillation.

[e] Averages significantly different at the 20% level.

Coastal Bermudagrass ensiled in the full-head stage than that harvested in the prehead stage. As neither the dry matter nor the fiber content was strikingly affected by stage of maturity, the explanation for this phenomenon must be found elsewhere. It has been observed that the lignin content does not increase rapidly as Coastal Bermudagrass grows older (5). With advancing age the physical properties change appreciably. The more mature Coastal Bermudagrass is tougher, more woody in appearance, and harder to cut finely, which results in more long pieces going through the forage harvester. Thus, it does not appear to pack as tightly as the younger material.

Average temperatures which developed during the ensiling process are presented in Figure 1. These were not greatly different in silages made from Coastal Bermudagrass at the two harvest stages. The peak temperature developed somewhat earlier in the less mature material. According to common belief, brown silage may result if the temperatures rise much above 100 F (2). Other deleterious effects of high temperature appear to be adequately proven (6, 15). These include increased fermentation, undesirable type fermentation, and the destruction of lactic acid and other desirable materials (15). Under practical conditions high temperatures are associated with increased nutrient losses. Also, it is commonly believed that the peak temperature should exceed certain minimums (2). Otherwise, it has been claimed, an undesirable fermentation producing a butyric acid type silage may result (2). However, very extensive studies of Nilsson et al. (15) do not indicate that a low temperature per se has deleterious effects. Rather, the undesirable preservation appears to be due to other factors, such as very high moisture levels.

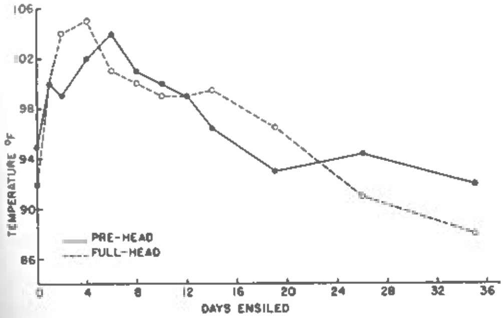


FIG. 1. Average temperatures in silos filled with Coastal Bermudagrass harvested at the prehead and full-head stages of maturity.

In determining the most suitable stage of maturity for harvesting Coastal Bermudagrass for silage, several factors are important, including the following: a). As presented above, nutrient losses were lower when the material was harvested at the prehead stage. b). The data on organic acids content suggest a more desirable fermentation for the forage harvested at the earlier stage. c). Coastal Bermudagrass harvested at an early age for hay or for pellets has been shown to result in high milk production, weight gains, and digestibility (8-10). d). Generally, dry matter yields per acre per season are higher when Coastal Bermudagrass is harvested at an older age (16). In the study reported herein, the yields of dry matter per harvest were 2,760 and 4,660 lb per acre for the prehead and full-head Coastal Bermudagrasses. e). The older material has a tendency to lodge, which increases the likelihood of clogging harvesting machinery. Also, a larger amount of material is not harvested as it goes under the sickle bar. This tends to become more erect after the tops are removed and may then be harvested with a subsequent cutting, substantially lowering its quality.

Additional studies with Coastal Bermudagrass for silage are needed to further delineate the factors influencing nutrient losses and quality changes. Experiments designed to determine why the loss of nutrients is increased with more mature Coastal Bermudagrass and to further explain its very unusual ensiling characteristics should be extremely interesting and useful.

ACKNOWLEDGMENTS

The authors thank Mr. J. Stone for technical assistance, Mrs. Rochelle Downing and Mr. J. Mason for chemical analyses, and Drs. E. R. Beaty and T. H. Rogers for evaluating stage of development and for agronomic advice.

REFERENCES

(1) ARCHIBALD, J. G., BLAISDELL, M. L., GERSTEN, B., AND KINSMAN, D. M. Grass Silage: A Reappraisal. Massachusetts Agr. Expt. Sta., Bull. 477. 1954.

(2) BRIGGS, R. A., LANGSTON, C. W., AND ARCHIBALD, J. G. Definition of Silage Terms. Agron. J., 53: 280. 1961.

(3) COLOVOS, N. F., KEENER, H. A., AND DAVIS, H. A. Errors in Drying Silage and Feces for Protein and Energy Determinations. Improved Procedures. J. Dairy Sci., 40: 173. 1957.

(4) GORDON, C. H., DERBYSHIRE, J. C., WISEMAN, H. G., KANE, E. A., AND MELIN, C. G. Preservation and Feeding Value of Alfalfa Stored as Hay, Haylage, and Direct-Cut Silage. J. Dairy Sci., 44: 1299. 1961.

(5) Knox, F. E., Burton, G. W., and Baird, D. M. Effect of Nitrogen Rate and Clipping Frequency upon Lignin Content and Digestibility of Coastal Bermuda Grass. Agr. and Food Chem., 6: 217. 1958.

(6) Langston, C. W., Irvin, H., Gordon, C. H., Bouma, C., Wiseman, H. G., Melin, C. G., Moore, L. A., and McCalmont, J. R. Microbiology and Chemistry of Grass Silage. USDA, Tech. Bull. 1187. 1958.

(7) Langston, C. W., Wiseman, H. G., Gordon, C. H., Jacobson, W. C., Melin, C. G., Moore, L. A., and McCalmont, J. R. Chemical and Bacteriological Changes in Grass Silage During the Early Stages of Fermentation. 1. Chemical Changes. J. Dairy Sci., 45: 396. 1962.

(8) McCullough, M. E., and Burton, G. W. How Fertilization, Cutting and Harvesting Affect Quality in Coastal Bermudagrass Hay. Georgia Agr. Research, 4: (No. 1). 4. 1962.

(9) Miller, W. J., Beaty, E. R., Clifton, C. M., and Brooks, O. L. Digestibility of Coastal Bermuda Pellets as Affected by Harvesting Age and Chemical Composition as Influenced by Season. Paper to be presented at the 58th Annual Meeting of the A.D.S.A., June, 1963.

(10) Miller, W. J., Brooks, O. L., Beaty, E. R., and Clifton, C. M. Effect of Frequency of Harvesting Coastal Bermudagrass for Pellets when Fed with High and Low Concentrates to Dairy Cows. Agron. J., 55: (In press.) 1963.

(11) Miller, W. J., Clifton, C. M., and Cameron, N. W. Nutrient Losses and Silage Quality as Affected by Rate of Filling and Soybran Flakes. J. Dairy Sci., 45: 403. 1962.

(12) Miller, W. J., and Dalton, H. L. Effect of the Addition of Citrolas to High-Moisture Forage for Silage on Nutrient Losses and Animal Response. J. Dairy Sci., 44: 1915. 1961.

(13) Miller, W. J., Dalton, H. L., and Miller, J. K. Immature Forage Mixtures with Citrus Pulp versus More Mature Forage Without Additive for Silage. J. Dairy Sci., 43: 993. 1960.

(14) Miller, W. J., Dalton, H. L., and Miller, J. K. Sudan Grass Silage at Two Stages of Maturity versus Rye Grass and Crimson Clover with Two Filling Procedures. J. Dairy Sci., 44: 1921. 1961.

(15) Nilsson, R., Toth, L., and Rydin, C. Studies on Fermentation Process in Silage. The Role of Temperature. Arch. Mikrobiol., 23: 366. 1956.

(16) Prine, G. M., and Burton, G. W. The Effect of Nitrogen Rate and Clipping Frequency upon the Yield, Protein Content and Certain Morphological Characteristics of Coastal Bermudagrass [*Cynodon dactylon*, (L) Pers.]. Agron. J., 48: 296. 1956.

(17) U. S. Department of Agriculture, Dairy Cattle Research Branch, Beltsville, Maryland. Summary of Second Silage Conference. 1959.

(18) Wiseman, H. G., and Irvin, H. M. Determination of Organic Acids in Silage. Agr. and Food Chem., 5: 213. 1957.

CARBON SOURCES FOR IN VITRO PROTEIN SYNTHESIS BY RUMEN BACTERIA [1, 2]

W. H. HOOVER,[3] E. M. KESLER, AND R. J. FLIPSE
Department of Dairy Science, The Pennsylvania State University, University Park

SUMMARY

In vitro tracer studies were conducted to determine the rate at which several volatile fatty acids and glucose were utilized by rumen bacteria, and the amount of carbon from these sources incorporated into bacterial proteins, nucleic acids, and polysaccharides. The rate at which each metabolite was utilized by rumen microorganisms was estimated on the basis of the decline in specific activity during incubation. The average rates calculated for acetate, propionate, butyrate, and valerate, in milligrams/liter/hour, were 131.0, 18.2, 13.2, and 0.6, respectively. Glucose was utilized in proportion to the amount added. At 10 and 20 mg/milliliter levels, 556 and 1,155 mg/liter/hour were utilized, respectively.

After incubation, some cellular components of the microorganisms were examined for C^{14} content. Nucleic acids contained label from acetate and glucose, but little or no activity from propionate, butyrate, or valerate. Activity from all labeled metabolites was found in the proteins. Of the activity that disappeared from the labeled acetate, propionate, butyrate, valerate, and glucose during incubation, the average amounts incorporated into proteins were 2.5, 2.1, 1.6, 2.6, and 2.9%, respectively. Cellular polysaccharides also became labeled from each source.

During studies on nitrogen metabolism in the rumen, it has been observed that certain carbohydrate sources enhanced or depressed the conversion of nitrogen to bacterial protein (8, 12, 15, 16). At present, however, only limited data are available on the conversion of various carbohydrate or other carbon sources to bacterial protein. McNaught (10) conducted an in vitro carbon balance study with rumen microorganisms using maltose, arabinose, and xylose as carbohydrate sources. The percentages of carbon from each of these sources found in bacterial proteins were 11.8, 16.7, and 16.1, respectively. Carbon from various other compounds has been found to be incorporated into bacterial amino acids. For example, Otagaki et al. (14) reported activity from C^{14}-labeled sodium carbonate, casein, glutamic acid, and leucine in both essential and nonessential amino acids of rumen bacteria incubated in vitro. More recently, Allison et al. (1) noted conversion of C^{14} from carboxyl-labeled isovaleric acid into the protein fraction of an isolated strain of rumen bacteria. The observation by Allison et al. suggests the possibility that other volatile fatty acids may be utilized as carbon sources for amino acid synthesis by rumen bacteria. It has been established that the volatile fatty acids in the rumen are further metabolized by the bacteria (6), but the extent of this process and the end products are largely unknown.

In the present study an attempt was made to estimate the relative rates at which acetate, butyrate, propionate, valerate, and glucose were utilized by rumen bacteria, and to study the possible incorporation of carbon from these compounds into bacterial protein.

EXPERIMENTAL PROCEDURE

Rumen fluid. The rumen fluid used throughout this work was from a mature Guernsey steer, equipped with a rumen fistula. The animal was maintained on a semipurified diet consisting of 46.3% oat straw, 15% cane molasses, 15% powdered dextrose, 15% corn starch, 3% lard, 4% urea, 1% dicalcium phosphate, 0.5% mineralized salt, and 0.2% cod liver oil. The

---

Received for publication September 24, 1962.

[1] Authorized for publication on September 5, 1962, as Paper No. 2702 in the Journal series of the Pennsylvania Agricultural Experiment Station.

[2] Data presented in this paper were taken from a thesis submitted by the senior author to the Graduate School in partial fulfillment of the requirements for the Ph.D. degree.

[3] Present address: Department of Animal Science, University of Maine, Orono.

oat straw was ground in a hammer mill and mixed with the other ingredients, and the diet was fed free choice during the experimental period.

Samples of rumen fluid for incubation were removed through a length of copper tubing inserted through the fistula. The tubing was connected to a two-liter flask which was equipped with a rubber squeeze-bulb to provide a vacuum.

Incubation procedure. The rumen fluid was prepared for incubation according to the procedure reported by McNaught (11). These authors state that the procedure removes protozoa; therefore, the organisms remaining are referred to as bacteria throughout the present paper. All incubations were carried out under CO_2 in either 125- or 500-ml Erlenmeyer flasks for periods of 6, 10, or 15 hr at 30 C, using 10 or 20 mg of glucose/milliliter as an energy source. The flasks were fitted with a two-hole rubber stopper. In one hole was inserted a short length of glass tubing, with a piece of rubber tubing on the end which could be clamped off. This inlet was used to flush the air from the flask with CO_2. The other hole contained a glass tube to which was attached a rubber balloon. The balloon served as an individual gas supply for each flask, and the resiliency of the balloon allowed changes in gas volume with little change in pressure. The CO_2 pressure in the balloons was adjusted to 8-10 mm Hg during the incubation period. Four incubations were conducted with each of the following C^{14}-labeled metabolites: uniformly labeled glucose, carboxyl-labeled acetate, and carboxyl-labeled butyrate. Three incubations were conducted with carboxyl-labeled propionate and three with carboxyl-labeled valerate. Ten to 20 μc of labeled compound were added to 200 ml of rumen fluid in each incubation, with the exception that in one incubation with each metabolite 50 μc of the labeled compound were added to 40 ml of rumen fluid. The bacteria from the entire 40 ml were harvested in the latter flasks, whereas in all other incubations 25-ml samples were taken from the flasks at various intervals throughout the incubation period. The pH of the incubation medium was adjusted hourly to 6.4-6.5 by additions of a few drops of saturated Na_2CO_3 solution.

Bacterial growth. Increases in bacteria during incubation were measured on the basis of increases in weight of the sludge harvested from the incubation flasks by centrifugation. The bacteria were initially harvested by centrifuging the 25-ml rumen fluid samples at 18,000 to 24,000 × G for 90 min at 10 C. The supernants were frozen for volatile fatty acid (VFA) and urea determinations. The precipitates were successively washed by centrifugation in 5% trichloroacetic acid (TCA) solution, absolute ethanol, and ether. The ether was driven off, leaving the dried precipitate, which was weighed. Increases in weight of the precipitate from 25 ml of rumen fluid over that from samples taken at the beginning of the incubation are reported as increases in bacteria.

DNA measurements. Comparisons of the levels of deoxyribonucleic acid (DNA) in the incubated samples with the DNA content of the same samples before incubation were used to substantiate the premise that weight increases observed in the bacterial harvesting procedure were the result of bacterial multiplication. The nucleic acids were extracted from the bacteria by the method of Schneider (17) and the DNA content was determined colorimetrically according to the method of Webb and Levy (20). The quantity of nucleic acid solution obtained in the extraction procedure was in excess of that needed for the colorimetric determination, and part of the remaining solution was plated onto stainless steel planchets in duplicate 500 λ samples for radioactivity determinations.

Bacterial protein determinations and radioactivity of bacterial protein. Estimates of the protein content were made from micro-Kjeldahl nitrogen determinations on the material obtained by centrifugation of the rumen fluid. Protein was calculated using N × 6.25. Samples for estimating the C^{14} incorporated into bacterial proteins were prepared by plating, onto planchets, known quantities of the hot TCA precipitate from the nucleic acid extraction. The planchets were dried under infrared heat lamps. In the trials in which 50 μc of each metabolite were incubated with 40 ml of rumen fluid, the bacteria were harvested from the total 40 ml of rumen fluid after 10 hr of incubation. These bacteria were hydrolyzed in 25 ml of 14% $Ba(OH)_2$ for 20 hr at 125 C. This resulted in a solution of bacterial amino acids and polysaccharides, which were separated by their relative solubilities in 80% ethanol. Samples of the polysaccharides and amino acids were plated onto planchets for radioactivity determinations.

VFA determinations. Aliquots of 1 or 2 ml were taken from the supernatant rumen fluid of the samples from the incubation flasks, and subjected to VFA analysis. The procedure used was that of Wiseman and Irwin (21), as modified by McCarthy (9). The acids were titrated, made alkaline, and evaporated to dryness. They then were dissolved in a known volume of 30%

ethanol and plated out in duplicate 500 λ aliquots for determination of radioactivity.

Glucose analysis. The amount of glucose present in the samples was determined by the reducing sugar method of Underkofler et al. (19) on samples of cell-free supernatant, deproteinized by the procedure of Doak (3). The specific activity (SA) of the glucose was determined by spotting 25 λ aliquots (in triplicate) of the deproteinized solution of known sugar concentration on Whatman No. 1 filter paper and chromatographing it by the method of French et al. (4). The resulting spots were located by Rf values, cut out, and eluted onto planchets.

Urea determinations. Urea levels of the rumen fluid were determined by the Van Slyke and Cullen method as outlined in Hawk, Oser, and Summerson (7).

Estimation of the utilization rates of the VFA. As mentioned previously, the incubation flasks were sampled periodically and the bacteria removed by centrifugation. The remaining supernatants were then examined for VFA concentration and SA. Along with the expected increase in VFA concentration and subsequent decline in SA, it was noted that the total activity per milliliter of supernatant rumen fluid (which resulted from the labeled metabolite) decreased throughout incubation. Since the only additions of fluid to the flasks were three or four drops of saturated Na_2CO_3 per hour, it was reasoned that the decrease in activity per milliliter reflected that portion of labeled metabolite utilized by bacteria. The decreases in activity per milliliter throughout the incubation periods are shown in Table 2. A rate constant for this decline was calculated according to the method suggested by Gray et al. (5). Because the VFA concentrations increased during incubation, it was necessary to modify the procedure. This was accomplished by determining the average concentration of VFA present during incubation and dividing it into the activity per milliliter present in the supernatant at each sampling period. The logs of these values, plotted against time, resulted in a line the slope of which reflected the amount of VFA removed or metabolized by the bacteria per unit of time. The difference between the log of the initial SA and log of the final SA was substituted into the following formula:

$$\text{Rate } (k) = \frac{1}{t} \left(\log_e \text{ initial SA} - \log_e \text{ final SA}\right)$$

This formula reduces to:

$$k = \frac{\text{difference in log SA}}{10} \times 2.3$$

The hourly rate of decrease, *k*, then was multiplied by the average concentration of the acid in the incubation flask to give the rate of utilization in milligrams.

Radioactivity determinations. All samples were placed in either aluminum or stainless steel planchets and counted in a thin-window, gas-flow Geiger-Muller detector.

RESULTS AND DISCUSSIONS

Bacterial growth and protein synthesis. Incubation periods of 10 hr were used most frequently throughout these trials. In this length of time some 40-50% of the glucose added as an energy source had disappeared, and an increase in bacterial weight of two to three times that originally present resulted. The average amount of urea present in the rumen fluid prior to incubation was 0.310 mg/milliliter. During incubation an average of 0.174 mg/milliliter of urea disappeared from the incubation flasks. Addition of urea before incubation was found to decrease bacterial growth.

Because bacterial growth was based on the increase in weight of the material harvested by

TABLE 1

Concentrations and rates of utilization of VFA and glucose by rumen microorganisms in vitro

Metabolite	No. of incubations	Avg concentration all samples mg/liter	Avg rate of utilization mg/liter/hour	Avg rate of utilization % utilized/hour
Acetate	3	4,070	131.0	3.2
Propionate	2	1,500	18.2	1.2
Butyrate	3	982	13.2	1.3
Valerate	2	144	0.6	0.4
Glucose	2[a]	10,000	556.0	5.6
Glucose	1[b]	20,000	1,155.0	5.8

[a] Glucose added to incubation flask at the rate of 10 mg/milliliter.

[b] Glucose added to incubation flask at the rate of 20 mg/milliliter.

TABLE 2

Incorporation of C^{14} from VFA and glucose into bacterial proteins and nucleic acids

Labeled metabolite used and incubation no.[a, b]	Activity of metabolite count/minute/milliliter		Final nucleic acid activity	Final bacterial protein activity	Final concentration of bacterial protein
	Initial	Final	*(count/min/mg of DNA)*	*(count/min/mg)*	*(mg/ml)*
Acetate					
1	37,100	27,200	3.2	280	0.57
2	35,100	26,500	2.3	250	0.60
3	70,800	54,000	5.1	880	0.80
Propionate					
1	75,800	68,000	0.0	110	0.72
2	70,500	61,400	T[c]	320	0.90
Butyrate					
1	11,200	9,700	T	27	0.62
2	12,800	10,500	T	40	0.67
3	15,300	13,900	T	45	0.73
Valerate					
1	52,500	41,200	T	280	0.58
2	41,500	38,000	T	180	0.76
Glucose					
1	49,200	30,000	87.2	1,080	0.62
2	60,300	25,600	32.8	1,300	0.71
3	44,000	15,000	75.1	840	0.88

[a] Sufficient labeled metabolite with a specific activity of 1-5 mc/mM was added in each case to give total added activity of 10-20 μc per flask.

[b] In addition to the labeled metabolite, all flasks contained 200 ml of clarified rumen fluid. All VFA incubations no. 1 contained 10 mg/milliliter glucose as an energy source. All VFA incubations no. 2 and 3, 20 mg/milliliter glucose. Glucose incubations 1 and 3, 10 mg/milliliter (unlabeled) glucose, incubation 2, 20 mg/milliliter (unlabeled) glucose.

[c] Trace amounts present.

centrifugation from rumen fluid, it was necessary to show that this weight increase was caused by an increase in bacterial numbers. Belozersky and Spirin (2) reported that under most conditions the DNA content of bacterial cells remains constant, except for a brief period before cellular division. As the number of cells increases, the total DNA content of the culture should increase in direct proportion. The nucleic acids were extracted from air-dry samples of material centrifuged from rumen fluid. The concentration of DNA was estimated colorimetrically, and the quantity was read in micrograms from a standard curve. The relationship between bacterial growth and DNA content is illustrated in Figure 1. The bacterial weights in Figure 1 represent several samples of bacteria centrifuged from rumen fluid after various periods of incubation. DNA content of these samples averaged 23 μg/milligram of air-dry material, and indicated that the increase in weight resulted from an increase in bacterial numbers.

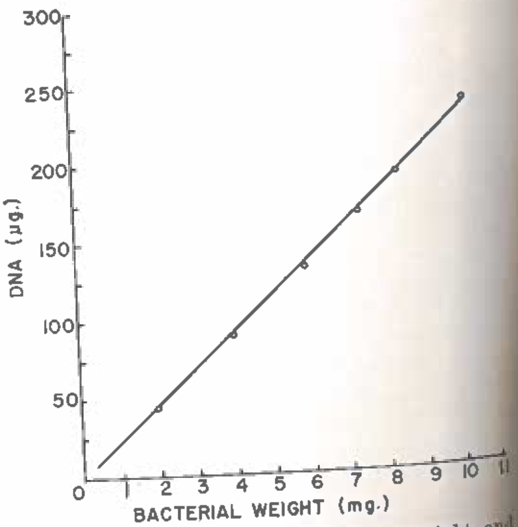


FIG. 1. Relation between bacterial weight and DNA content.

Micro-Kjeldahl analysis of the material centrifuged from rumen fluid and dried at 100 C for 5 hr revealed a decrease in crude protein content during incubation. Samples taken prior to incubation averaged 61% crude protein, and after 10 hr of incubation the average was 36%. These findings are similar to those reported by Smith and Baker (18). They found that bacterial weight increased from 1 to 4 g/liter after a 4-hr incubation, but the protein content of the bacteria decreased from 50 to 33%. In the present study there was an average increase in bacterial weight of 2.5× in 10 hr. With the corresponding decrease in protein content, the net synthesis of protein averaged 0.22 g/liter.

Utilization of glucose and VFA. Rates of bacterial utilization of the various labeled compounds were calculated. When labeled glucose was added to the incubation flasks the rate of glucose utilization could be deduced from the decrease in activity of the glucose per milliliter of rumen fluid, since the total concentration of glucose decreased at an equal rate. The utilization of VFA by the microorganisms could not be measured in this way, because VFA are continually being produced in vitro, at a faster rate than they are being utilized. This results in a greater decrease in specific activity of the labeled VFA than can be attributed to turnover rate alone. Since the method used to calculate the rate at which the microorganisms utilized VFA was based on the decrease in specific activity of the VFA during incubation, it was necessary to reduce the influence of excess microbial production of VFA on decline of specific activity. To accomplish this the specific activity of the fatty acid was adjusted by using an average concentration of the fatty acid during the incubation period. For example, the concentrations of butyric acid as removed from the Wiseman columns were 212, 212, 216, and 240 mg/liter for the sampling times 0, 3, 6, and 10 hr, respectively. This was an average of 220 mg of butyric acid/liter. In arriving at the adjusted specific activities for butyrate at the various sampling periods, the counts/minute/liter resulting from butyric acid were divided by the average number of milligrams of butyric acid/liter, or 220. When the logs of the adjusted specific activities were plotted against time they gave a straight line. Plots of adjusted specific activities for each fatty acid appear in Figures 2 and 3. The highest and lowest specific activities for each incubation were then inserted into the formula:

$$k = \frac{1}{t} (\log e \text{ initial SA} - \log e \text{ final SA})$$

where k = the rate constant in terms of the fraction of the amount present that disappears in unit time. The value for k in the case of butyric acid was:

$$k = \frac{1}{10} (2.3) (4.1430 - 4.1038)$$

$$k = 0.009$$

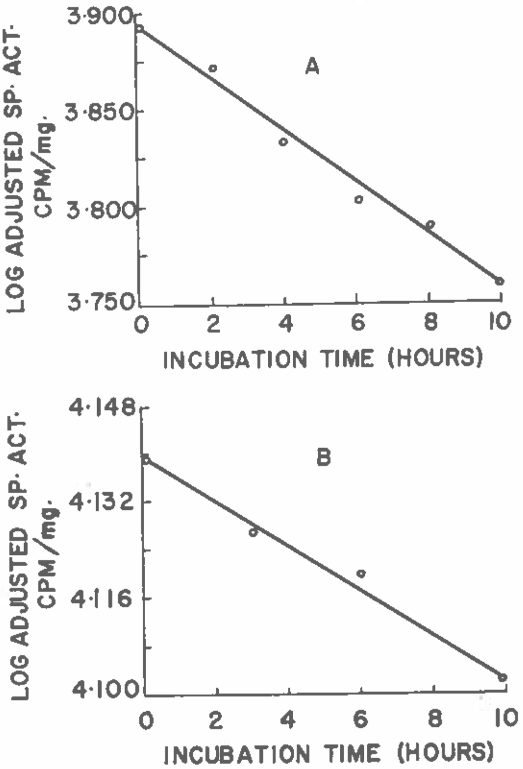


FIG. 2. Rate of loss of activity of labeled acids in vitro. A—acetate incubation, Flask 1; B—butyrate incubation, Flask 3.

The rate constant then was multiplied by the average concentration of butyrate present in the incubation flask throughout the incubation:

$$0.009 \times 1{,}100 \text{ mg/liter} = 9.9 \text{ mg butyrate utilized per hour by the bacteria in one liter of flask contents.}$$

Calculations of the disappearance, on a liter basis, for all fatty acids and glucose appear in Table 1. This table also contains the average concentrations of each VFA and glucose found in all incubations. Rates of utilization appear to reflect concentrations of each metabolite. The rate of glucose utilization was increased markedly by raising the concentration of glucose in the medium, but the influence of changing the concentration of VFA was not investigated. The figures in Table 1 indicate that under the conditions of these experiments, in the period of one hour, bacteria utilized 163 mg of VFA per liter of rumen fluid.

Incorporation into protein and nucleic acids. The incorporation of the carbon from the

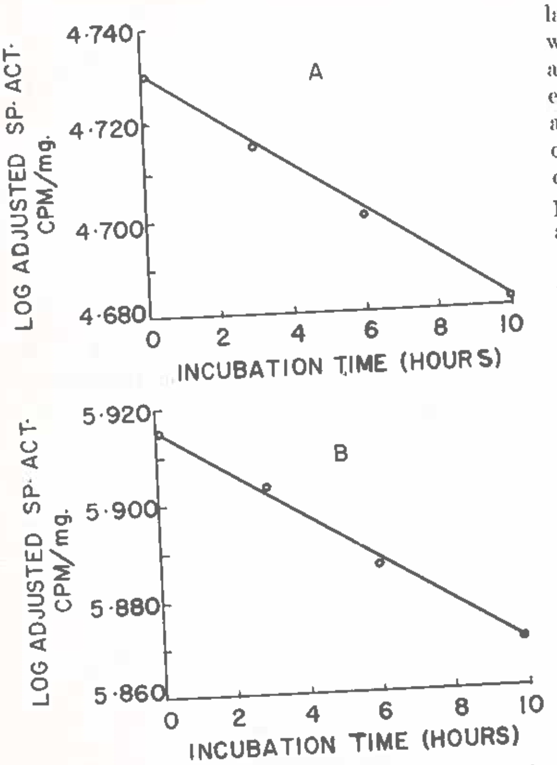


FIG. 3. Rate of loss of activity of labeled acids in vitro. A—propionate incubation, Flask 1; B—valerate incubation, Flask 1.

labeled metabolites into bacterial protein and nucleic acids, as well as an indication of the amounts of labeled compounds that disappeared from the rumen fluid during the incubation periods, is indicated in Table 2. Of the activity that disappeared during incubation, only a small portion was located in the cellular components studied. Nucleic acid activity is based on planchets containing 30-40 μg DNA, plus an unmeasured amount of RNA. Activity is reported as a trace when the number of counts on a sample were between one and two times background. The bacterial protein activity is based on gross measurement of the activity in the precipitate from hot TCA. The concentration of bacterial protein per milliliter of rumen fluid is derived from the total weight of precipitate from the rumen fluid at the end of incubation, multiplied by the average protein content, 36%. Only glucose and acetate contributed appreciably to nucleic acid synthesis. The VFA acetate, propionate, and valerate gave similar protein activities at the end of the incubations, while butyrate carbon was incorporated to a lesser extent. The percentages of the activity which disappeared from the labeled metabolites during incubation, and which were found in bacterial protein were acetate 2.5, propionate 2.1, butyrate 1.6, valerate 2.6, and glucose 2.9. When the percentages for VFA are multiplied by the milligrams of each VFA utilized by bacteria in 10 hr, the combined VFA converted to protein per liter per 10 hr was 39 mg, of which 85% was from acetate.

No attempt was made to account for all of the activity which disappeared during incubation. It is assumed that at least part of it escaped as CO_2.

Throughout the work, activity in bacterial proteins was estimated on residues from hot TCA hydrolysis. This TCA precipitate analyzed 76-78% crude protein on a N × 6.25 basis. The 22-24% contamination was thought to be bacterial polysaccharide, and it was necessary to determine whether the activity in polysaccharides was contributing greatly to the specific activity of the TCA precipitate. To investigate this, an incubation was conducted with each metabolite, using 50 μc of activity and 40 ml of rumen fluid. The microorganisms were removed by centrifugation and hydrolyzed in a base to prevent polysaccharide degradation. After hydrolysis the amino acids were separated from the polysaccharides by their relative solubilities in 80% ethanol. The polysaccharides were suspended in a known volume of water, ranging from 6.3 to 7.2 ml, and heated to 80 C. This caused most of the polysaccharides to go into solution. The amino acid solutions were desalted by the procedure of Mueller et al. (13), evaporated to dryness, and taken up in a known volume of water. The activity in both solutions was measured and is presented in Table 3. Most of the activity was found in the amino acid fractions of the microorganisms in each incubation. With the exception of the glucose incubation, the activity in the polysaccharides was low. It was concluded

TABLE 3

Relative C^{14} activities in the polysaccharide and amino acid fractions of hydrolyzed bacteria

Labeled metabolite	Total activity in polysaccharides	Total activity in amino acids
	(count/min)	*(count/min)*
Acetate	4,060	66,700
Propionate	3,530	42,000
Butyrate	3,450	30,000
Valerate	1,170	8,000
Glucose	39,400	71,000

that the activity found in the residues from the hot TCA precipitate resulted mainly from the protein in these fractions.

Results of these investigations indicate that, under these experimental conditions, each of the metabolites studied was utilized by rumen microorganisms. The rates of utilization varied widely, glucose being metabolized in larger quantities than volatile fatty acids. Of the fatty acids studied, the rates of utilization were proportional to their concentration in the incubation flasks. Carbon from each of these sources was incorporated into the amino acids and polysaccharides of the rumen bacteria and, to a smaller extent, into the nucleic acids.

REFERENCES

(1) Allison, M. J., Bryant, M. P., and Doetsch, R. N. Conversion of Isovalerate to Leucine by *Ruminococcus flavefaciens.* Arch. Biochem. Biophys., 84: 245. 1959.

(2) Belozersky, A. N., and Spirin, A. S. Chemistry of the Nucleic Acids of Microorganisms. p. 147-161. *In* The Nucleic Acids, Vol. III. Chargaff, E., and Davidson, J. N., eds. Academic Press, Inc., New York and London. 1960.

(3) Doak, B. W. A New Method for Clarification of Plant Extracts for the Determination of Reducing Sugars. New Zealand J. Sci. Technol., 21: 90-B-5B. 1939.

(4) French, D., Knapp, Doris W., and Pazur, J. H. Studies on the Schardinger Dextrins. VI. The Molecular Size and Structure of the γ-Dextrin. J. Am. Chem. Soc., 72: 5150. 1950.

(5) Gray, F. V., Jones, G. B., and Pilgrim, A. F. The Rates of Production of Volatile Fatty Acids in the Rumen. Australian J. Agr. Research, 11: 383. 1960.

(6) Gray, F. V., Pilgrim, A. F., Rodda, H. J., and Weller, R. A. Fermentation in the Rumen of Sheep. IV. The Nature and Origin of Volatile Fatty Acids in the Rumen of Sheep. J. Exptl. Biol., 29: 57. 1952.

(7) Hawk, P. B., Oser, B. L., and Summerson, W. H. Practical Physiological Chemistry. 13th ed. p. 552-553. Blakiston Co. Inc., New York. 1954.

(8) Houpt, T. R. Utilization of Blood Urea in Ruminants. Am. J. Physiol., 197: 115. 1959.

(9) McCarthy, R. D. Tracer Studies of the Perfused Rumina and Livers of Goats. Ph.D. thesis, University of Maryland. 1958.

(10) McNaught, Mary L. The Utilization of Non-Protein Nitrogen in the Bovine Rumen. 7. A Qualitative and Quantitative Study of the Breakdown of Carbohydrate which Accompanies Protein Formation in Bovine Rumen Contents During *in Vitro* Incubation. Biochem. J., 49: 325. 1951.

(11) McNaught, M. L., Smith, J. A. B., Henry, K. M., and Kon, S. K. The Utilization of Non-Protein Nitrogen in the Bovine Rumen. 5. The Isolation and Nutritve Value of a Preparation of Dried Rumen Bacteria. Biochem. J., 46: 32. 1950.

(12) Mills, R. C., Lardinois, C. C., Rupel, I. W., and Hart, E. B. Utilization of Urea and Growth of Heifer Calves with Corn Molasses or Cane Molasses as the Only Available Carbohydrate in the Ration. J. Dairy Sci., 27: 571. 1944.

(13) Mueller, G. C., Bowman, Grace, and Herranen, Ailene. Desalting Amino Acid Solutions by Ion Exchange. Anal. Chem., 27: 1357. 1955.

(14) Otagaki, K. K., Black, A. L., Goss, H., and Kleiber, M. *In Vitro* Studies with Rumen Microorganisms Using Carbon-14-Labeled Casein, Glutamic Acid, Leucine, and Carbonate. Agr. Food Chem., 3: 948. 1955.

(15) Pearson, R. M., and Smith, J. A. B. The Utilization of Urea in the Bovine Rumen. 3. The Synthesis and Breakdown of Protein in Rumen Ingesta. Biochem. J., 37: 153. 1943.

(16) Reis, P. J., and Reid, R. L. *In Vitro* Studies on the Effect of pH and of Glucose on Ammonia Accumulation in the Rumen of Sheep. Australian J. Agr. Research, 10: 71. 1959.

(17) Schneider, W. C. Phosphorus Compounds in Animal Tissues. I. Extraction and Estimation of Desoxypentose Nucleic Acid and of Pentose Nucleic Acid. J. Biol. Chem., 161: 293. 1945.

(18) Smith, J. A. B., and Baker, F. The Utilization of Urea in the Bovine Rumen. 4. The Isolation of the Synthesized Material and the Correlation Between Protein Synthesis and Microbial Activities. Biochem. J., 38: 496. 1944.

(19) Underkofler, L. A., Guymon, J. F., Rayman, M. M., and Fulmer, E. I. A Semi-Micro Method for the Determination of Reducing Sugars in Fermentation Media. Iowa State Coll. J. Sci., 17: 251. 1943.

(20) Webb, J. M., and Levy, H. B. A Sensitive Method for the Determination of Desoxyribonucleic Acid in Tissues and Microorganisms. J. Biol. Chem., 213: 107. 1955.

(21) Wiseman, H. G., and Irwin, H. M. Determination of Organic Acids in Silage. Agr. Food Chem., 5: 213. 1957.

EFFECTS OF FEEDING DIETHYLSTILBESTROL ON THE DEVELOPMENT AND REPRODUCTIVE PERFORMANCE OF DAIRY CATTLE

L. J. BUSH AND H. W. REUBER[1]
Departments of Dairying and Physiology, Oklahoma State University, Stillwater

SUMMARY

The effects of continuous feeding of diethylstilbestrol (DES) on the development and reproductive performance of dairy cattle were determined.

Heifers fed DES starting at four months of age made more rapid weight gains ($P < 0.05$) to 15 months of age than controls; however, the difference between groups diminished thereafter. Differences in mammary gland development and height of tailhead were small and temporary in nature. DES feeding had no effect on the ability of the heifers to conceive and produce a normal calf.

There was some indication that reproduction was impaired in a few cows fed 15 mg DES daily starting 30 days postpartum; however, the results with mature cows were inconclusive.

As a result of the widespread use of diethylstilbestrol (DES) in rations for fattening beef cattle, interest has developed concerning the possible effects of feeding DES to dairy cattle of different ages.

Krawczyk and Olson (3) reported that feeding heifers 10 mg of DES daily starting at four to 14 months of age increased weight gains and wither height during a 210-day period. On the other hand, Fosgate and Hedge (2) reported that feeding 10 mg of DES daily increased the rate of gain of heifers over a 58-day period in one trial, but not in another. Rumery and Ward (5) found no significant increase in weight gain or wither height from feeding heifers DES at the rate of 10 mg daily for 351 days. Voelker (7) observed no increase in the growth of dairy calves resulting from feeding 2 or 3 mg of DES daily from four to 116 days of age.

Mammary gland development has been reported by several workers (3-5) to be similar in DES-fed and control heifers. In contrast, Voelker (8) reported that heifers implanted with 15 mg DES monthly for four successive months developed asymmetrical udders.

Krawczyk and Olson (3) observed DES-treated and control heifers to have essentially the same number of estrous cycles, differing only slightly in length. Reuber (4) observed no effect from DES on the frequency and duration of estrus in yearling beef heifers; however, in another trial involving heifers started at seven months of age, those fed DES had over twice as many heat periods as did untreated controls. No significant difference in the number of services required for conception has been observed (3-5) between heifers fed DES and controls. Similarly, Wrenn and Sykes (9) and Browning et al. (1) reported that feeding DES at levels from 5-15 mg daily for varying periods of time had no adverse effect on reproduction in dairy cows.

Previous work on feeding DES to dairy heifers involved animals of varying ages or sizes (3, 5) and relatively short experimental periods (2, 3, 5, 7). Therefore, this study was conducted to determine the effects of continuous feeding of DES on the development and reproductive performance of dairy heifers from four months of age to parturition. In addition, further information was obtained concerning the effect of DES feeding on reproduction in lactating dairy cows.

EXPERIMENTAL PROCEDURE

Two trials were conducted using Holstein cattle from a brucellosis-free herd, in which the calves were vaccinated for brucellosis at seven months of age.

In the first trial, 20 heifer calves were paired on the basis of date of birth and started on experiment at four months of age. One member of each pair was individually fed DES in the grain at the rate of 5, 10, and 15 mg per day from four to six, six to 12, and 12 months of age to calving, respectively. Mammary gland development was evaluated by monthly measurements of teat length to 15 months of age

Received for publication March 27, 1963.

---

[1] Present address: Pharmacology Department, Salsbury's Laboratories, Charles City, Iowa.

and udder width and length to eight months of age, using the procedure described by Swett et al. (6). Body weight and height of tailhead were recorded at monthly intervals to 18 months of age. The height of tailhead was determined as the distance from pinbone to pinbone in a vertical plane over the tailhead.

Observations were made for estrus and inclination of heifers not in estrus to mount other animals at approximately 8-hr intervals. All heifers were bred artificially starting with the first estrous period after 15 months of age.

In a second trial, 20 cows were paired on the basis of freshening date. Beginning at 30 days postpartum one member of each pair was fed 15 mg of DES daily until the end of the subsequent gestation, except for cows not conceiving during a five-month breeding period. All cows were bred artificially at each estrous period starting at 60 days postpartum, and DES feeding was discontinued for cows not pregnant by seven months postpartum. In cases where pregnancy did not ensue after a four-month period without DES feeding, the cows were slaughtered to ascertain the cause of infertility.

RESULTS AND DISCUSSION

About ten days after the start of the experiment, edema of the vulva and perineum was noted in the heifers fed stilbestrol. This condition, also previously noted by other workers (1, 3) did not persist, but recurred at irregular intervals throughout the experiment. The average increase in height of tailhead was slightly greater in the heifers fed DES than in controls. However, the difference between groups, although statistically significant ($P < 0.01$), was not great enough to be detected visually and was considered to be of no practical importance.

Only slight differences were found between the two groups in width and length of udder during the period from four to eight months of age, and there was no noticeable difference between the two groups in appearance of the udders at parturition. The apparent edema of the udder and increased teat length observed during the early part of the experiment (Figure 1) was temporary in nature and evidently of little significance. Moreover, the relatively small, yet statistically significant ($P < 0.01$), difference between groups in teat length disappeared at about 12 months of age, coinciding with the onset of puberty (Figure 2). Thus, it appeared that DES feeding had no permanent effect on udder development of dairy heifers, which is in agreement with results obtained by other workers under similar experimental conditions (3-5).

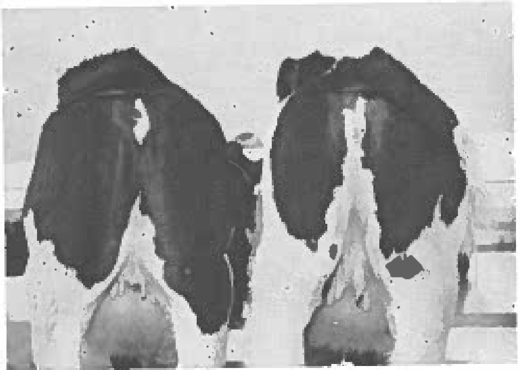


FIG. 1. Comparison of control heifer (left) with heifer fed 5 mg DES daily for 6 wk (right).

The average weight gain of the heifers to 15 months of age was significantly greater ($P < 0.05$) for the DES-fed group than for controls (Table 1). However, the difference between groups diminished during the next three months, possibly because of a change in hormone balance brought about by pregnancy in most of the heifers at this period.

Although the heifers fed DES attained puberty 35 days earlier on the average than those in the control group, the difference between groups was not statistically significant ($P > 0.10$). Likewise, the number and length of estrous cycles were not sufficiently different for the two groups to indicate any marked change in ovarian function of the DES-fed heifers. The most noticeable difference between the two groups was the increased aggressiveness of the heifers fed DES, as evidenced by the number of attempts made by animals not showing clinical signs of estrus to mount other heifers (Table 1).

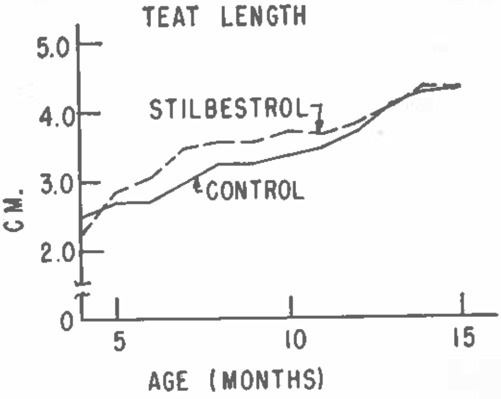


FIG. 2. Average teat length of heifers from 4 to 15 months of age.

TABLE 1

Growth and reproductive performance of heifers

Measurement	Stilbestrol	Control
Weight gain *(lb)*		
4-15 months [a]	529	471
4-18 months	650	620
Age at puberty *(days)*	360	395
Number of estrous cycles prior to first breeding at 15 months of age	4.8	3.4
Length of estrous cycles prior to first breeding *(days)*	21.6	25.9
Aggressiveness rating [a, b]	17.5	8.4
Services per conception	1.5	1.9

[a] Difference between groups statistically significant ($P < 0.05$).

[b] Average number of times heifers not in heat were observed to mount others from 12 to 15 months of age.

There was essentially no difference between groups of heifers as to their ability to conceive and produce a normal calf. The greater number of services required per conception for the control group was largely attributable to one animal which required an unusually large number of services. In each group, seven of the ten animals conceived at first service. The sex of the calves was apparently not affected by DES feeding. There were seven heifers and four bulls produced by the DES group, as compared to eight heifers and two bulls in the control group. Thus, continuous feeding of DES had no significantly adverse effects on either the development or reproductive performance of the heifers from four months of age to parturition.

Less consistent results were obtained with mature cows fed 15 mg DES daily from 30 days postpartum to the end of the subsequent gestation. Some cows fed DES conceived readily and calved normally. On the other hand, two cows in the DES group were slaughtered because of failure to conceive following several services during DES feeding and also during a subsequent period when the DES was withdrawn. The specific cause of infertility in these cows was not evident on gross and microscopic examination of the genitalia; however, factors other than DES feeding may have contributed to the condition as both of these cows had placental dystocia following the previous calving. Additional work with a larger number of cows would be needed to critically evaluate the effects on reproductive performance of continuous DES feeding at the rate of 15 mg daily for ten months or longer.

ACKNOWLEDGMENT

The diethylstilbestrol used in this work was generously provided by Eli Lilly and Company, Indianapolis, Indiana.

REFERENCES

(1) Browning, C. B., Marion, G. B., Fountaine, F. C., and Gier, H. T. Effect on Reproduction of Feeding Diethylstilbestrol to Dairy Cattle. J. Dairy Sci., 42: 1351. 1959.

(2) Fosgate, O. T., and Hedge, K. S. The Effects of Feeding Low Rates of Phenothiazine and Diethylstilbestrol to Young Dairy Heifers. J. Dairy Sci., 45: 293. 1962.

(3) Krawczyk, G. R., and Olson, H. H. Effect of Feeding 10 mg Stilbestrol Daily on Growth and Breeding Efficiency of Dairy Heifers. J. Dairy Sci., 44: 1188. 1961.

(4) Reuber, H. W. Effects of Diethylstilbestrol Feeding on the Bovine Reproductive Tract. Am. J. Vet. Research, 19: 585. 1958.

(5) Rumery, M. G. A., and Ward, G. M. The Influence of Stilbestrol with a High Roughage Ration in Raising Replacement Dairy Heifers. J. Dairy Sci., 44: 1199. 1961.

(6) Swett, W. W., Book, J. H., Mathews, C. A., and Fohrman, M. H. Evaluation of Mammary-Gland Development in Holstein and Jersey Calves as a Measure of Potential Producing Capacity. USDA, Tech. Bull. 1111. 1955.

(7) Voelker, H. H. Stilbestrol—How Does It Affect Young Dairy Calves? South Dakota Farm and Home Research, 8: 3. 1957.

(8) Voelker, H. H. Effects of Diethylstilbestrol Implants on Milk Production. South Dakota Farm and Home Research, 11: 7. 1959.

(9) Wrenn, T. R., and Sykes, J. F. Effects of Feeding Stilbestrol to Lactating Dairy Cows. J. Dairy Sci., 40: 1581. 1957.

TECHNICAL NOTES

ELECTRON MICROSCOPIC OBSERVATIONS OF ALKALINE EARTH METAL-CASEINATE PARTICLES

It has been well established that casein exists in milk as roughly spherical particles of varying sizes (1, 3, 7, 8) and containing calcium and magnesium (4). The effects of the metals on the particle formation and size distribution in complexing with casein have stimulated further interest in the behavior of the caseinate system. This paper presents evidence that when alkaline earth metal chlorides are added to casein solutions, the size of the particles depends upon the particular alkaline earth metal added.

Suspensions of the alkaline earth metal-caseinates were prepared by adding the following amounts of 0.1 M alkaline earth metal chloride solutions to 100 ml of 0.01% casein solutions: 0.5 ml of barium chloride, 0.25 ml of beryllium chloride, 1 ml of calcium chloride, 1 ml of magnesium chloride, 0.25 ml of strontium chloride. They were allowed to stand for 2 hr. The casein was obtained from cow's milk according to the directions of Warner (10) and dissolved in 0.01 N sodium hydroxide solution, then adjusted to pH 7.0 with dilute hydrochloric acid. A small drop of water (2 mm in diameter) containing the alkaline earth metal-caseinate was placed in the center of collodion-coated copper grids and allowed to air-dry. After drying, a heavy coating of carbon was evaporated almost vertically onto the grids, giving mechanical strength to the preparation. The preparation was then shadowed with a small piece of chromium at an angle of about 30°. Electron micrographs were taken by Hitachi electron microscope type HW-1, using an accelerated voltage of 50 kv.

When a certain amount of the alkaline earth metal chloride was added to the sodium caseinate solution, spherical particles were observed in the electron micrographs (Figure 1). Since the preparation of sodium caseinate did not give any regularly shaped particles, it is evident that the arrangement is due to the reaction between casein and the metal chloride.

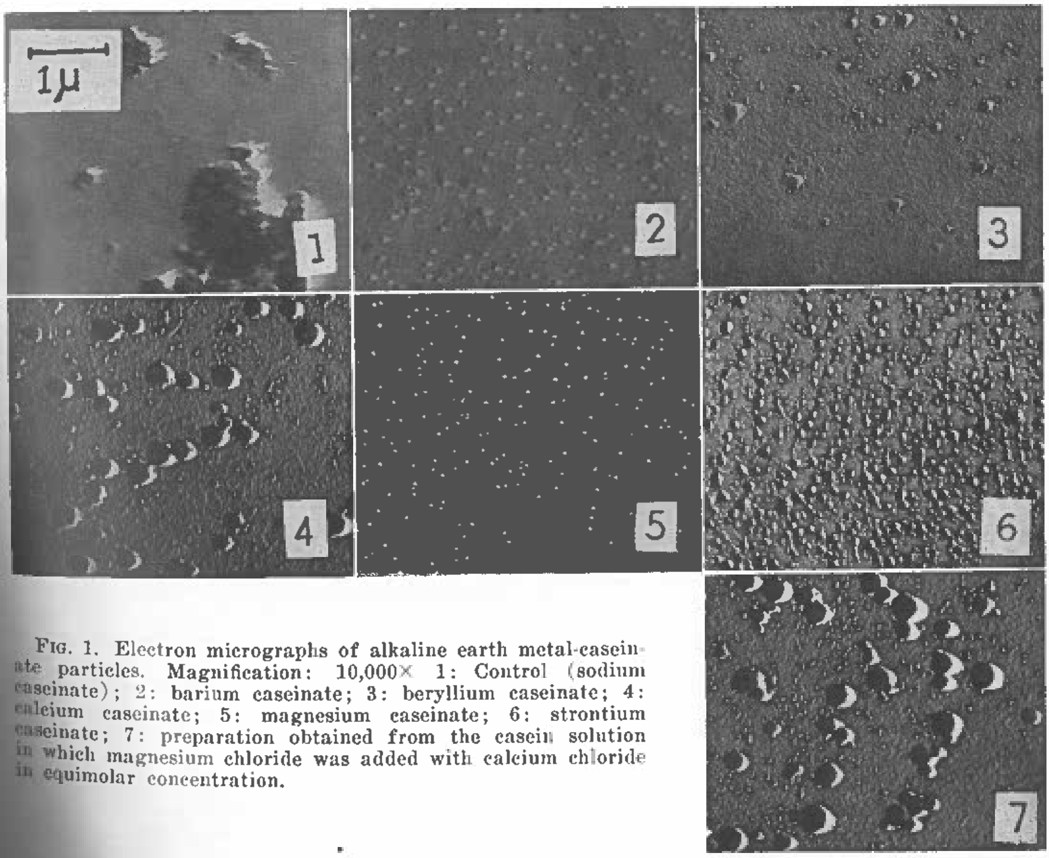


FIG. 1. Electron micrographs of alkaline earth metal-caseinate particles. Magnification: 10,000× 1: Control (sodium caseinate); 2: barium caseinate; 3: beryllium caseinate; 4: calcium caseinate; 5: magnesium caseinate; 6: strontium caseinate; 7: preparation obtained from the casein solution in which magnesium chloride was added with calcium chloride in equimolar concentration.

It should be mentioned that, in such preparations, these alkaline earth metal chlorides do not interfere with observation, since they crystallize on drying and areas free of crystals are frequently found. However, when an excess of these chlorides was used, one could not clearly observe the individual particles, as the material in the electron microscope field appeared amorphous. The diameter of the particles of these caseinates is generally as follows: barium caseinate, 50-90 mμ; beryllium caseinate, 50-200 mμ; calcium caseinate, 80-400 mμ; magnesium caseinate, 20-40 mμ; strontium caseinate, 30-150 mμ. The size of this calcium caseinate is in agreement with that found by Kiyosawa, Sadachika, and Maeno (5).

The values obtained with the different caseinates do not fall off in the order of the size of the metal ions. It can be seen that the formation of the largest size of caseinate particles occurred in the addition of calcium ion, which occupies the mean position in the series of magnitude for the atomic weight of the alkaline earth metals. Since some of the alkaline earth metals are known to complex preferentially with ligends (2, 6, 9), it is possible that the metals in the particles are associated with various sites of the caseinates and the affinity of the metals for a given site is considerably different. When 1 ml of the magnesium chloride solution was added to 100 ml of the casein solution, together with 1 ml of the calcium chloride solution, all of the particles resembling calcium caseinate were present and no particles resembling magnesium caseinate were present. Therefore, it appears that the possibility of contamination of magnesium caseinate particles with the calcium caseinate particles is questionable.

ACKNOWLEDGMENT

Thanks are due to Mr. Tsuruji Satoh for the technical assistance.

SUSUMU ADACHI
Laboratory of Animal Products Technology
College of Agriculture
Tohoku University, Sendai, Japan

REFERENCES

(1) DJATSHENKO, P., SCHDANOWA, E., AND POLUKAROWA, JU. Study of Milk with the Aid of the Electron Microscope. Mol. Prom., 16(5): 35. 1955.

(2) GURD, F. R. N., AND WILCOX, P. E. Complex Formation Between Metallic Cations and Proteins, Peptides, and Amino Acids. Adv. Protein Chem., 11: 311. 1956.

(3) HOSTETTLER, H., AND IMHOF, K. Electronenoptische Untersuchungen über die submikroskopische Struktur von Milch und Milcherzeugnissen. Landwirtschsft. J. Schweiz, 66: 309. 1952.

(4) JENNESS, R., AND PATTON, S. Principles of Dairy Chemistry. John Wiley & Sons, Inc., New York. 1959.

(5) KIYOSAWA, I., SADACHIKA, K., AND MAENO, M. Studies on the Physical and Chemical Properties of Casein. I. A Study on the Structure of Casein Micelles in Milk. J. Zootech. Soc., 29: 33. 1958.

(6) KLOTZ, I. M. Thermodynamic and Molecular Properties of Some Metal-Protein Complexes. *In* The Mechanism of Enzyme Action. (McElroy, W. D., and Glass, B., eds.) Johns Hopkins Press, Baltimore. 1954.

(7) KNOOP, E., AND WORTMANN, A. Zur Grössenverteilung der Caseinteilchen in Kuhmilch, Ziegenmilch, und Frauenmilch. Milchwissenschaft, 15: 273. 1960.

(8) NITSHMANN, Hs. Elektronenmikroskopische Grössenbestimmung der Calciumcaseinatteilchen in Kuhmilch. Helv. Chim. Acta, 32: 1258. 1949.

(9) SCHWARZENBACH, G. The Specificity of Metal Complex Formation. *In* Chemical Specificity in Biological Interactions. (Gurd, F. R. N., ed.) Academic Press, New York. 1954.

(10) WARNER, R. C. Separation of α- and β-Casein. J. Am. Chem. Soc., 66: 1725. 1944.

POTENTIOMETRIC DETERMINATION OF SALT IN CHEESE

The procedure commonly used to determine salt in cheese (1) is a modification of the Volhard technique, including the wet-ashing procedure of Davies (2). This procedure has many obvious disadvantages, i.e., large amounts of highly toxic fumes are produced, many operations are involved, and an excess of an expensive reagent, $AgNO_3$, is employed. Silverman et al. (4) simplified the standard procedure by eliminating filtration through the use of nitrobenzene. A potentiometric method for the determination of NaCl in milk, published by Herrington and Kleyn (3), suggested further simplification of salt analysis for cheese.

MATERIALS AND METHODS

A Beckman pH meter, model H2, was used as the potentiometer and a silver electrode was prepared as described by Herrington and Kleyn (3). The reference electrode was a Beckman saturated calomel electrode. Preliminary experiments showed that a sharper change in potential is obtained at the end point at approximately pH 2.0, a fact also observed for milk by Herrington and Kleyn (3). The end point for cheese, as for milk, was found to be +255 mv with respect to the saturated calomel electrode.

Approximately 2 g of cheese, previously

finely chopped, was weighed into 250-ml beakers. To each beaker was added 100 ml of distilled water. The mixture was brought to a boil, cooled, and titrated with N/10 $AgNO_3$. Later, it was found unnecessary to boil the mixture, to get results comparable with the standard method. If the cheese only rests in contact with water at 60 C for at least 15 min, good results are obtained. To avoid the necessity of adjusting the pH to 2.0 after extracting, the water was replaced by a dilute solution of HNO_3. A solution containing 1.5 ml of concentrated HNO_3 per liter gave a pH of 2.0 in the final solution. Use of a no. 1 cork borer to obtain samples, rather than cutting portions, eliminates mincing prior to weighing. Since one borerful gives about 0.3 g of cheese, five or six plugs from different parts of the cheese will give a good representative sample.

EXPERIMENTAL PROCEDURE

1. Grind cheese finely (unnecessary if using a no. 1 cork borer).
2. Weigh accurately approximately 2.0 g in 250-ml beakers.
3. Add 100 ml of a dilute HNO_3 solution.
4. Place beakers in a water bath at about 60 C for at least 15 min.
5. Titrate when convenient with standardized $AgNO_3$ until a difference of +255 mv is obtained between the silver electrode and the saturated calomel electrode. This is the end point.
6. Calculate the per cent NaCl as usual.

For best results, stir with a magnetic stirrer during titration.

The result of salt analysis on a number of cheeses of various varieties is shown in Table 1. Triplicate samples were run in all cases. Closer agreement was obtained between samples of the same cheese using the potentiometric method than with the standard method. This is probably due to the easier reproduction of the end point.

TABLE 1

Salt analysis on different types of cheese by the standard method and the potentiometric method

Cheese	A.O.A.C. method	Potentiometric method
	(% NaCl)	
Young Cheddar (1 wk old)	2.165	2.165
	1.395	1.400
	1.630	1.725
	2.140	2.200
	2.330	2.340
	2.164	2.163
Mature Cheddar	1.241	1.239
Processed Cheddar	1.570	1.614
Blue	4.177	4.192
Swiss	0.526	0.565
	1.753	1.714
	0.877	0.838
	1.462	1.423
	0.468	0.420
	2.018	2.028

A Sargent Automatic Titrator equipped with a commercial silver electrode also has proven satisfactory.

P. F. Fox
Department of Dairy and Food Science
Cornell University, Ithaca, New York

REFERENCES

(1) Association of Official Agricultural Chemists. Official Methods of Analysis of the A.O.A.C. p. 210. Washington, D. C. 1960.

(2) Davies, W. L. The Determination of Chloride in Dairy Products and Biological Material. Analyst, 57: 79. 1932.

(3) Herrington, B. L., and Kleyn, D. H. A Potentiometric Method for the Determination of Chloride in Milk. J. Dairy Sci., 43: 1050. 1960.

(4) Silverman, G. J., Wolin, A. G., and Kosikowski, F. V. Simplification of the Standard Method for Salt Analysis in Cheese. J. Dairy Sci., 42: 1095. 1959.

INEXPENSIVE TECHNIQUE FOR PRODUCTION OF C^{14} ALFALFA FOR ANIMAL FEEDING EXPERIMENTS[1]

C^{14}-labeled alfalfa could serve as a useful tracer in rumen metabolism studies, but it is not available commercially. Preparation of labeled plant material requires a special growth chamber in which $C^{14}O_2$ of high specific activity is utilized in the photosynthetic process. Several such apparatuses have been constructed (1, 4, 5), but their use is limited because of high construction costs or small capacity. This paper describes a growth chamber for biosynthesis of labeled plant material for rumen metabolism studies at a low cost.

MATERIALS AND METHODS

The growth chamber (Fig. 1) was constructed of $\frac{3}{8}$-in. clear Plexiglas[2] held to-

---

[1] Contribution No. 399, Department of Bacteriology; No. 316, Department of Dairy Science; and No. 827, Department of Agronomy, Kansas Agricultural Experiment Station, Manhattan.

[2] Rohm & Haas Inc., Washington Square, Philadelphia 5, Pennsylvania. In this manuscript trade names are used for clarity. Their use does not imply endorsement or that competitive products are not equal.

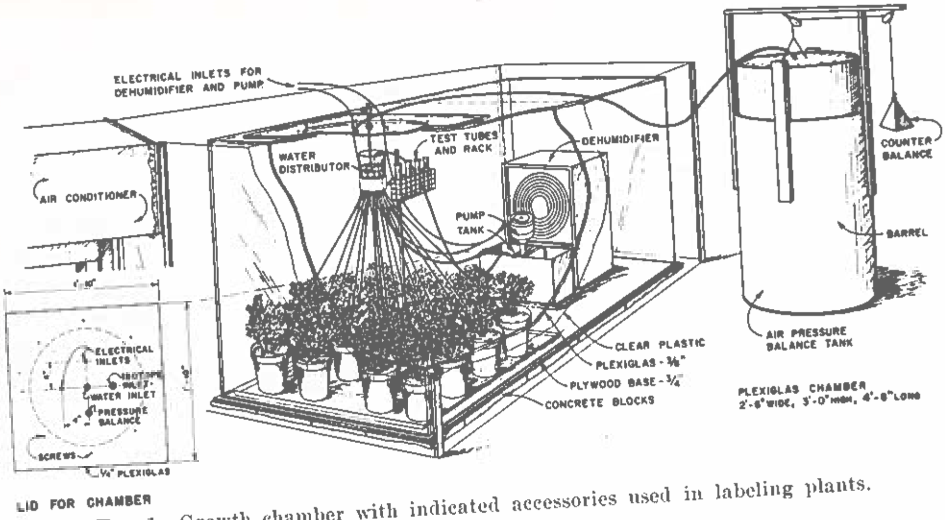


FIG. 1. Growth chamber with indicated accessories used in labeling plants.

gether by brass machine screws (10 × 24 × ¾ in.) at 2-in. intervals. The Plexiglas edges were softened with ethylene dichloride before being fastened. Even though this formed airtight joints, all seams were calked on the inside with a nonhardening calking compound as a precaution against leakage.

An access porthole (18 in. in diameter) in the top of the chamber was closed by a Plexiglas lid 22 in. square by ¼ in. thick. The lid was held in place by eight ¾-in. screws that passed through the lid and screwed into the chamber top. To form an airtight seal nonhardening calking compound was used.

A dehumidifier[3] was placed in the chamber through the porthole and mounted on a base of plywood and polystyrene blocks to absorb vibration. The relative humidity in the chamber was maintained at 55-60% to prevent fungal growth at higher humidities.

Water condensed by the dehumidifier was recirculated to the plants by an electric vertical immersion pump placed on the collection tank. The pump was activated, when necessary, by a switch placed outside the chamber. Water was pumped through a ⅝-in. plastic tube into a one-liter container supported on a ring stand and mounted above the plants. A No. 15 rubber stopper served as the base of the container. Sixteen 4.5-in. sections of ¼-in. copper tubing extended through holes in this stopper. They were connected by rubber tubing to $\frac{3}{16}$-in. diameter glass tubes positioned over each plant. Distribution of water was controlled by preset screw cocks. When additional water was required, it was passed into the dehumidifier tank through a glass stopcock in the lid, then pumped to the plants. When excess water accumulated, it was siphoned out through the same stopcock. Electrical wires to the dehumidifier and pump entered the chamber through rubber stoppers inserted in the lid.

Pressure changes within the chamber due to temperature and barometric fluctuations were absorbed by a spirometer consisting of a 45-gal airtight drum inverted over 50 gal of water layered with 1.5 in. of mineral oil. Air passed from the chamber to the spirometer through a polyethylene tube extending through a rubber stopper in the lid. The inverted drum was held in place during ascent and descent by four wooden guides placed around the circumference of the outer barrel. Pressure created in raising the drum was counterbalanced by a weight and pulley arrangement. The drum seldom moved more than 8 to 10 in. during labeling runs.

The growth chamber was maintained in a conventional greenhouse. To prevent excessive temperature variations a canopy of clear Flex-O-Glas[4] was built around the chamber. It extended about 6 in. from all sides and the top, excluding the access portion. An air conditioner was positioned to blow into the canopy. On extremely hot days a cheese cloth shade was pulled over the top. This kept the interior of the chamber below 80 F, even when the greenhouse temperature rose to 100 F.

Total cost of the chamber was approximately $400, including labor, all parts, accessories, and dehumidifier but excluding the air conditioner.

The alfalfa plants were obtained from a spaced-plant nursery and transferred while dormant to 6-in. pots. Soon after growth was initiated, 16 plants treated with an insecticide were placed into the chamber, on a grill supported by pans, to allow for good drainage. Before the plants were placed into the chamber, a complete nutrient solution was provided. Artificial light was used to provide an 18-hr day.

---

[3] Westinghouse, Model No. ESC 70002.

[4] Warp Bros., 1100 N. Cicero Ave., Chicago 51, Illinois.

A measured amount of $BaC^{14}O_3$ was placed in test tubes and mixed with distilled water. The tubes were held in a rack fastened to the ring stand, and placed directly under a hole in the lid. The hole was plugged by a rubber serum stopper. To release the $C^{14}O_2$ from the $BaC^{14}O_3$, 5 ml of 85% phosphoric acid was injected through the serum stopper by a long needle attached to a syringe. Levels of C^{14} activity were monitored by withdrawing 10 ml of the chamber atmosphere with a gastight syringe. The CO_2 was absorbed in hydroxide of Hyamine 10-X[5] and counted in a liquid scintillation spectrometer.

Plants were removed at the early-bloom stage and placed in the dark (usually for 48 hr) to metabolize reserve sugars. They were then harvested, placed in paper bags, and dried at 100 to 120 F in a forced draft oven for 48 hr.

Cellulose was extracted by the method of Crampton and Maynard (3). The C^{14} activity of the total plant material and the cellulose was determined by the wet combustion method of Claycomb et al. (2).

RESULTS AND DISCUSSION

The quantities of C^{14} recovered in the dry weight of the total plant material and cellulose from several labeling runs are shown in Table 1. On the average, 33.8% of the isotope released was recovered in the plant tissue. The greatest recovery of C^{14} was in the healthiest and most vigorously growing plants. The per cent of C^{14} recovered in plant material was similar, whether all the $C^{14}O_2$ was released at periodic intervals during growth or only once (Table 1). Losses in the oil or the water used in the spirometer were insignificant. After 60 mc of C^{14} had been released in the chamber, the counts on the water and mineral oil were 5 to 10 per milliliter above background, or approximately 1 μc in all of the water and oil in the barrel.

While the original purpose for which alfalfa was prepared did not require a high degree of uniform labeling, it should be possible to obtain uniformly labeled plant material by using stricter control measures. Nonuniform distribution of radioactivity in plants may be due to a number of factors. The $C^{14}O_2$ may be diluted by soil CO_2, and unlabeled carbon present in the roots may be translocated to the first few inches of new growth. Material with greater C^{14} activity was obtained when plants were placed in the chamber early and when the same plants were used repeatedly to benefit from root-stored C^{14}. The use of hydroponic cultures should increase uniformity of labeling.

SUMMARY

An inexpensive growth chamber for labeling alfalfa with C^{14} for rumen metabolism studies is described. Approximately 34% of the C^{14} released in the chamber was recovered in the plants. The chamber could be used for labeling other plants in either soil or hydroponic culture.

C. L. Keith
E. E. Bartley
E. L. Sorensen
L. R. Fina
and
D. W. Stanton
Departments of Bacteriology, Dairy Science, Agronomy, and Crops Research Division, ARS, USDA
Kansas State University
Manhattan, Kansas[6]

TABLE 1

C^{14} recovery in harvested alfalfa (dry matter basis) from five labeling runs

Weight of plant material	Cellulose	Isotope released	Total C^{14}	Recovered cellulose C^{14}	Isotope recovered
(g)	(%)	(mc)	(mc)	(mc)	(%)
84.0	40.9	5.0	1.682	0.919	33.6 [a]
32.6	32.2	10.0	2.838	1.000	28.3 [a]
71.5	39.2	10.0	4.090	1.640	40.9 [b]
49.5	36.4	10.0	3.342	1.459	33.4 [b]
52.5	42.4	7.9	2.601	1.002	32.8 [b]

[a] One release.
[b] Four releases.

REFERENCES

(1) Brown, J. C., Tiffin, L. O., and Holmes, R. S. Carbohydrate and Organic Acid Metabolism with C-14 Distribution Affected by Copper in Thatcher Wheat. Plant Physiol., 33: 38. 1958.

(2) Claycomb, C. K., Hutchens, T. T., and Van Bruggen, J. T. Techniques in the Use of C^{14} as a Tracer. 1. Apparatus and Technique for Wet Combustion of Non-volatile Samples. Nucleonics, 7: 38. 1950.

---

[5] Packard Instrument Co., Inc., Box 428, La Grange, Illinois.

[6] Supported in part by funds provided by Regional Project NC-63.

(3) CRAMPTON, E. W., AND MAYNARD, L. A. The Relation of Cellulose and Lignin Content to the Nutritive Value of Animal Feeds. J. Nutrition, 15: 383. 1938.

(4) FISHER, A. C., JR. You and the Obedient Atom. Natl. Geog. Magazine, 114: 303. 1958.

(5) SCULLY, N. J., CHORNEY, W., KOSTAL, G., WATANABE, R., SKOK, J., AND GLATTFELD, J. W. Biosynthesis in C^{14}-Labeled Plants; Their Use in Agricultural and Biological Research. Proc. Intern. Conf. Peaceful Uses Atomic Energy, 12: 377. 1955.

OATS, RYEGRASS, AND CRIMSON CLOVER FOR YOUNG DAIRY ANIMALS[1]

Winter temporary forages continue to be widely grown in the South. Their feeding value has been extensively demonstrated by research and is common knowledge to dairymen. The literature reveals that little attention has been given to using these forages for growing dairy heifers.

Florida (3) and Georgia (4) research has shown excellent rates of gain and growth of heifers on winter pasture alone. Satisfactory growth of heifers on pasture crops alone to first calving, at a considerable saving over conventional methods, has been reported by Louisiana workers (7). McCullough et al. (5) found a high correlation between dry matter digestibility and average daily gain ($r = 0.796$) and indicated that forage quality, among several cool-weather species, was very similar and did not limit animal gains until the grasses began to mature (4).

Oats, ryegrass, and crimson clover were investigated, to compare the species individually and in mixtures for young dairy animals.

EXPERIMENTAL PROCEDURE

Two one-acre lots were seeded to each forage or forage combination as follows: (1) oats, 4 bu; (2) ryegrass, 44 lb; (3) oats, 3½ bu and ryegrass, 22 lb; and (4) oats, 3 bu, ryegrass, 22 lb, and crimson clover, 30 lb. Fertilization practices were those recommended for the area by station agronomists. Since the lots were not irrigated, the amount and distribution of rain in the fall determined the earliest date they could be grazed.

Guernsey and Holstein heifer and steer calves, born in August and September, were used. They were well-suited to evaluate the forages for wintering replacement heifers or for bull calves for slaughter. Before the grazing trials, they were fed to maintain normal growth. The feeding regime consisted of milk or milk replacer for 42 days, a simple grain ration as soon as they would consume it, and hay and silage ad libitum.

During the grazing trials the forages constituted their total diet except for a salt-mineral mixture available throughout the trials. Both breeds were allotted equally among the forage treatments. Their average weights at the beginning of the trials were 175, 237, and 146 lb for the 1957-58, 1958-59, and 1959-60 seasons, respectively. Beginning weights were recorded seven days after the grazing trials had begun, to eliminate weighing error due to differences in fill. One-day weights were recorded at 14-day intervals. The system of grazing consisted of four animals per forage treatment grazed continuously on the same lots and extra animals rotated among all lots to provide uniform grazing conditions. In the second and third years, dry matter digestibilities were determined at 6-wk intervals by the chromogen technique of Reid et al. (6).

RESULTS AND DISCUSSION

Table 1 summarizes the main results. Average dry matter digestibilities were as follows: oats, 73%; ryegrass, 73.9%; oats + ryegrass, 71.4%; and oats + ryegrass + crimson clover, 74.1%. Analysis of variance showed no significant differences in average daily gain among the forage treatments. The animals were removed from the pastures in late spring before forage maturity would have resulted in lower digestibility and less favorable weight gains. There was a highly significant between-years difference for the 1958-59 season. That year, dry weather delayed grazing of the forages until January 28. The animals were gaining at a slower rate before the trial than in the other 2 yr and apparently made compensatory growth on the winter forages. This is in agreement with findings by Kincaid et al. (2) and Beeson et al. (1).

Total gain per acre varied significantly between years and treatments. The long 1959-60 season gave average gain per acre (all-forage average each year) of 751 lb, compared with 546 and 549 lb the two previous seasons. The oats + ryegrass + crimson clover mixture was significantly higher in gain per acre than the other forage treatments in all 3 yr. In gain per acre, the forages were quite consistent with oats + ryegrass + crimson clover highest, followed by ryegrass alone, oats + ryegrass, and oats alone. Although not statistically significant, their rank in gain per acre was prob-

---

[1] Georgia Experiment Station, Journal Series 448.

TABLE 1

Calf days per acre, average daily gain, and gain per acre for the four forages

Season		Calf days	Gain	Per acre avg daily gain
		(no.)	*(lb)*	*(lb)*
		Ryegrass alone		
1957-1958		313	545	1.74
1958-1959		195	544	2.79
1959-1960		394	793	2.01
	Avg	300	627	2.09
		Oats alone		
1957-1958		231	384	1.66
1958-1959		251	527	2.10
1959-1960		356	532	1.49
	Avg	279	481	1.72
		Oats + ryegrass		
1957-1958		273	481	1.74
1958-1959		253	532	2.10
1959-1960		368	676	1.83
	Avg	298	563	1.89
		Oats + ryegrass + crimson clover		
1957-1958		441	786	1.78
1958-1959		272	582	2.14
1959-1960		530	1,003	1.89
	Avg	414	790	1.91

Grazing periods: 1957-1958, December 9 through May 12; 1958-1959, January 28 through May 7; 1959-1960, November 11 through May 2.

ably consistent enough to indicate true differences.

The data demonstrate the value of selecting species for mixtures which are both high in feeding value and complementary in growth habits. The oats provided grazing in the early part of the grazing season and the ryegrass during the latter part. Thus, total production was increased when the two were used together, compared with oats alone.

Like most winter grazing crops these forages were highly digestible and supported a high rate of gain equally well, leaving total dry matter produced (and thus, total gain per acre) as the more critical factor in assessing their value for young dairy animals. Future work should be aimed at increasing per acre production of forage so long as it does not reduce quality.

L. R. Sisk
and
M. E. McCullough
Georgia Experiment Station
Experiment

REFERENCES

(1) Beeson, W. M., and Perry, T. W. Wintering—Pasturing—Fattening Steers—1950-1951. Indiana Agr. Expt. Sta., Mimeo. AH 74. 1954.

(2) Kincaid, C. M., Litton, C. W., and Hunt, R. E. Some Factors that Influence the Production of Steers from Pasture. J. Animal Sci., 4: 164. 1945.

(3) Marshall, S. P. Value of Oat Pasture for Dairy Cattle. Florida Agr. Expt. Sta., Bull. 584. 1957.

(4) McCullough, M. E., Baird, D. M., and Sell, O. E. The Relationship Between Forage Dry Matter Digestibility and Weight Gain by Dairy Heifers on Three Winter Grazing Crops. J. Dairy Sci., 37: 1435. 1954.

(5) McCullough, M. E., and Neville, W. E., Jr. Some Factors Affecting Weight Gains of Dairy Heifers Fed All-Roughage Rations. J. Dairy Sci., 42: 1698. 1959.

(6) Reid, J. T., Woolfolk, P. G., Hardison, W. A., Martin, C. M., Brundage, A. L., and Kaufmann, R. W. A Procedure for Measuring the Digestibility of Pasture Forage Under Grazing Conditions. J. Nutrition, 46: 255. 1952.

(7) Southeast Louisiana Dairy and Pasture Experiment station. Ann. Progr. Rept. 1961.

OUR INDUSTRY TODAY

MANAGEMENT PROGRAM BASED ON COMPARATIVE COST ANALYSIS[1]

FRED H. H. CALHOUN
Dairy Service, Incorporated
Lexington, North Carolina

In order for management to cope with the present accelerated pace of business, it must be properly informed. The heart of any management program based on a comparative cost analysis is a good cost accounting system. While it is true that the number of office workers has increased in this century five times faster than the labor force, it is not necessarily true that management is receiving five times as much information. Most of the increase in the labor cost of administration is due to the many reports that are required by the regulatory agencies and the complications of the payroll and tax reports. The comptroller is usually more occupied with meeting report deadlines than furnishing management with the necessary cost information.

REQUIREMENTS OF A COST ACCOUNTING SYSTEM

The cost accounting system must be capable of rendering accurate information with a minimum of expense. It must be adaptable to the complex dairy industry, be flexible enough to fit machine as well as hand accounting systems, and should be projectable into future operations and conditions. Every function of a dairy plant must be separated into an operation, with a separate profit and loss statement made for each department. An example would be the ice cream manufacturing department or the fluid milk department. Then each operation should be subdivided into comparable functions or cost centers, such as raw materials, labor, transportation, etc.

USING THE COST ACCOUNTING SYSTEM FOR FULL BENEFIT

To fully utilize a cost accounting system, a progressive management should join a cost comparison group. This would enable the management to make cost comparisons with others, and generally locate those areas where the most improvement is needed. Improvement is a never-ending process and by comparison we can constantly measure it and thereby keep pace with others. A good accounting system gives a cost for each operation and function. If the necessary comparisons are to be made comparable, the costs can be based on the sales dollar or the hundredweight processed. These are good measures but each have their weaknesses. For example, hundredweight sold and hundredweight processed are seldom the same. The number of week ends in a month mean little to a manufacturer, but a lot to a distributor, whereas the cost of a container means little to sales but a lot to a manufacturer. We can add a third dimension which will compensate for the shortcomings of per cent of sales and hundredweight by expressing product manufacturing and product distributing in a ratio of actual cost to a group standard. In Table 1 it can be seen that this new dimension adds depth to the comparison. Operation A has a manufacturing cost of $1.82 per hundredweight and an efficiency of 105%, whereas Operation B has a manufacturing cost of $1.26 per hundredweight and an efficiency of 104%. The relatively high distribution cost of 27% of the sales dollar in Operation D is just as efficient as the second lowest distribution cost of 15% of the sales dollar in Operation B. There are many other interesting aspects of this added dimension, but it does not tell us if the operation is at full capacity or if there is a good reason why one segment is lagging.

STANDARDS FOR MEASURING OPERATION EFFICIENCY AND COSTS

Standards for group comparison need to be fixed. However, an operation's ability to meet a fixed standard varies with volume and change in facilities. If the volume processed drops, total cost will also drop, but the cost per hundredweight will rise and the ability to meet a fixed standard will become impossible. Therefore, individual standards for a given operation need to be flexible in order to measure the obtainable efficiency of an operation. This can be accomplished by installing a flexible budget based on fixed and variable expenses, because it lets all department heads know what is expected and gives them obtainable goals. Another important product of a good comparative cost accounting system is the platform cost of each item manufactured. With the total direct cost of the function concerned and the allocations from other functions such as the service group, it is a rela-

---

[1] Presented to the Dairy Manufacturing Extension Section at the 57th Annual Meeting of the American Dairy Science Association, University of Maryland, College Park, June, 1962.

TABLE 1

Three-factor comparison of milk operation

	Dairy A			Dairy B			Dairy C			Dairy D			Average		
	Per cent of sales	Cost per cwt	Comp. to std.	Per cent of sales	Cost per cwt	Comp. to std.	Per cent of sales	Cost per cwt	Comp. to std.	Per cent of sales	Cost per cwt	Comp. to std.	Per cent of sales	Cost per cwt	Comp. to std.
Sales															
Retail	12			19			38			50			30		
Wholesale	39			26			55			42			40		
Other	49			55			7			8			30		
Total	100	9.68		100	8.49		100	10.80		100	11.26		100	10.06	
Product cost	63	5.94	98.9	66	5.42	98.7	57	5.95	99.1	55	5.84	97.2	60	5.79	98.5
Spread	37	3.74		34	3.07		43	4.85		45	5.42		40	4.27	
Manufacturing	18	1.82	105	14	1.26	104	18	2.03	77	13	1.57	92	16	1.67	95
Distribution	12	1.22	115	15	1.35	89	19	2.14	122	27	3.26	89	18	1.99	99
Total operating expense	30	3.04	104	29	2.61	93	37	4.17	90	40	4.83	82	34	3.66	92
Profit	7	.70		5	.46		6	.68		5	.59		6	.61	

tively simple matter to distribute the cost of the function to the units concerned. Two very important parts of platform costs are return on investment and administration. A 6% return on investment for manufacturing equipment is not excessive and is generally required in order to keep the equipment up to date. The unit cost should receive an administrative charge to cover office staff payroll and management supervision. During some months plant problems may require little time; during other months they may require the major portion of management's time. Where branch operations are involved, the cost of transportation of products to the branch should be charged to all products. The true platform cost is the total of all functions concerned, return on investment, administrative expense, and branch delivery cost. It is this cost, with the added profit desired, that is the true platform cost.

DISTRIBUTION COST STATEMENTS

Quarterly route profit and loss statements are the backbone of analyzing distribution costs. The largest route sales volume does not necessarily indicate the highest profit. Driving habits, vehicle abuse, and route returns play an important part in the real profit. Once platform costs are established, route profit and loss statements are relatively easy to obtain. Units will have to be recapped by routes and individual vehicle costs will have to be kept. Administrative, advertising, and supervisory expenses will have to be allocated. The results will far outweigh the cost of obtaining the quarterly route profit and loss statements.

OPERATION PROJECTION

One of the more important uses of cost accounting is to predict the profitability of a change in operations. If the operation is to be merged with another similar operation, what will the new costs be? What will be the effect of a government contract bid on costs? A management program based on comparative cost analysis will help solve these problems. To explain the use of cost accounting in predicting the future, the following is assumed:

1. The plant is operating below its capacity.
2. The plant is to bid on a government contract for 600,000 lb per month in half-gallon cartons.
3. The present volume is 1,250,000 lb per month.

The manufacturing functions that are affected by the projected change are receiving, processing, half-gallon packaging, and case handling. In Table 2 it can be seen that in the combined function of receiving and processing the cost per hundredweight will drop just under 11½¢ per hundredweight. Since the plant is processing 12,500 hundredweight per month, the cost of processing the present vol-

TABLE 2

Change in plant cost due to added volume

Paper	Receiving and processing	Packaging	Container and closure	Case handling	Total	Return on investment	Adm. and general	Product	Total
Dollars charged	$5,525	$1,330	....	$2,590	....	$3,085	$2,800	....	....
½-Gallon homo	.01900	.01330	.02450	.00647	.06327	.01061	.00963	.27735	.36087
With new volume	.01410	.00732	.02450	.00532	.05124	.00717	.00651	.27735	.34227
Savings per ½-gallon	.00490	.00598	....	.00114	.01202	.00345	.00312	....	.01859
Savings per present cwt	.11400	.04784	....	.03704	.19888	.08000	.07260	....	.35148
Total savings on present volume	$1,425	$598	....	$463	$2,486	$1,000	$908	....	$4,394

Distribution cost with added volume

Type of sales	Sales	Labor		Vehicle	Adver-tising	Operating expense	Adm. and general	Return on investment	Total	Cost per unit	Total cost of a ½-gallon delivered	
		Direct	Indirect								Pres. vol	New vol
Dollars charged		$8,198	$2,459	$6,968	$2,732	$2,595	$6,335	$683	$29,970			
Wholesale sales $.47/½-gal. 290,697 units	$136,628	6.0%	1.8%	5.1%	2.0%	1.9%	4.6%	0.5%	21.9%	.10310	.46397	.44537
Contract sales $.36/½-gal. 140,000 units	$50,400	Direct 1.0%	Included in direct	3.0%	0	0.5%	0.5%	0.2%	5.2%	.01872		.36099
Contract dollars		$504	0	$1,512	0	$252	$252	$101	$2,621			

Change in profit due to new volume

New volume unit cost	Bid price	Unit difference	Dollar difference	Volume savings	Increase in present profit
.36099	.36099	....	....	4,394	4,394
.36099	.35099	.01000	$1,400	4,394	2,994
.36099	.34099	.02000	$2,800	4,394	1,594
.36099	.32962	.03137	$4,392	4,394	2

ume will be lowered by $1,425. In the half-gallon packaging function there is a savings of $598. With the increase in volume the platform cost of each half-gallon is lowered by almost 2¢ per unit. The total savings in the manufacturing division is $4,394. The distribution costs of delivering the half-gallon cartons for the contract will be reduced almost 8½¢ per unit. There is a $4,394 recovery of overhead in the operation if the contract is bid at the delivery cost of $0.36099. If the bid is above the delivered cost, there will be more profit. It is possible to bid less than the platform cost and still have an increase in total profit. Cost accounting has done its job, and it is up to management to make a decision on the actual bid price.

SUMMARY

A management program based on cost analysis will aid any management in reaching a better decision. Five applications of cost accounting as a management tool are:

1. A comparison with other operations, to determine if the operation is just drifting or making real progress.
2. A comparison of the operation to its own flexible standards to determine if the performance is up to capabilities.
3. A platform cost for each item manufactured.
4. An individual route profit and loss statement.
5. An accurate method by which the future costs of an operation under varying conditions can be projected.

Information alone will not insure an efficient operation unless it is properly applied. Cost accounting makes no decisions. It may point out that several items are being sold below cost or that a particular route is losing money. It is up to management to determine why the cost of manufacturing is high and it must analyze the figures to determine why it is losing money. Cost accounting is a tool of management and not a substitute for management. It is up to the man in management who is responsible for the proper application of this tool to make the right decision.

Publication limitations did not allow the printing of a sample budget, unit cost data, route profit and loss statement, or volume cost graphs. These may be obtained on request from the author.

ASSOCIATION AFFAIRS

Memorial

Frank Victor Burcalow

Frank Victor Burcalow was born on a farm near Albany, Wisconsin, March 19, 1909. He graduated from high school at Monroe, Wisconsin, in 1927 and entered the University of Wisconsin in September of that year. He received the B.S. degree in Agronomy in 1932, with a minor in Agricultural Education. He operated the family dairy farm until 1935, when he became an Assistant in Agronomy in the University. He was an instructor in Agronomy, 1938-1941 and earned the M.S. degree in 1939. He continued in the Department of Agronomy and was appointed Professor in 1949. He held this position until his death at home, September 29, 1962.

F. V. Burcalow

In 1937 he married Lois Arlene Benkert. They had two children, Vicki Claire, now Mrs. Kent Johnson of Middleton, and Larry, at home, a sophomore in the University.

Professor Burcalow's principal interest was in the production and utilization of forage crops, especially as related to Wisconsin dairy farming. He was concerned also with problems of soil and water conservation in relation to farming practice. In cooperation with his colleagues, he strove to promote interest in reducing production costs of milk and other livestock products. He was interested also in the use of grasses for turf purposes.

In 1937 he was a United States representative to the International Grassland Congress at Aberystwyth, Wales. October, 1944, to December, 1945, he served with the Agricultural Division of the Allied Control Commission in Rome as an Agronomist consultant and in 1946 represented the Italian Government at the European Food Conference in London. In the period 1942-49 he was a turf consultant of the U.S. Department of Agriculture and gave special attention to airport and military base sod requirements. He was a member of the working committee to develop plans for the VIth International Grassland Congress held at Pennsylvania State University in 1952 and was Chairman of the Midwest Tours Committee of the Congress.

He became a member of the American Society of Agronomy in 1936, and was actively interested in forage crop and pasture research and served on many committees. He was concerned also with extension education and methodology. He was a member of the Society's Fertilizer Advisory Committee to F.A.O. of the United Nations. In 1953 he was made an Honorary Fellow of the American Society of Agronomy. He was a member of the Wisconsin Academy of Arts and Sciences and of the American Dairy Science Association.

Professor Burcalow was very active in organizations concerned with grassland agriculture in Wisconsin. From 1946 until his death he was Chairman of the College of Agriculture Grassland Farming Committee and of the Grassland Contest Committee, beginning in 1950. He was very instrumental in the development, promotion, and success of Farm Progress Field Days. He was one of the early extension workers to recognize the value of interdepartmental group action in the implementation of extension programs.

He wrote numerous extension bulletins, circulars, and farm paper articles pertinent to his field. With his long residence in Wisconsin and extensive travel as an Extension Specialist, he gained many friends among farmers and in business and industry.

Professor Burcalow participated in the community affairs of Middleton and took considerable satisfaction from his efforts. He was a member of the Middleton Civics Club as early as 1948 and was on the Park Board from 1947 until his death. In 1949 he helped to organize the Community Council. He was a charter member of the Middleton Sportsmen's Club, which he helped to organize in 1946, and was the club secretary-treasurer.

Professor Burcalow was known to be an enthusiastic crusader for objectives and purposes in which he was interested. He was effective in organization and execution of plans and was persistent in accomplishment. He was frank and outspoken, but liberal and tolerant. His early passing leaves a void which will not soon be filled, for many Wisconsin and other friends and co-workers.

BUSINESS MANAGEMENT OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

R. E. Hodgson, E. L. Jack, G. H. Wise, H. F. Judkins,
E. O. Herreid, and W. V. Price, Chairman,
Advisory Committee on Business Management

An advisory Committee on Business Management was appointed by the Executive Board in June, 1960, to study existing and future management needs of the Association. This summary of the committee's report to the Executive Board has been prepared to answer requests of members for more information on Item 23 of the Board's actions in June, 1961 (J. Dairy Sci., 44:1583. 1961.), when the report was considered.

The constitution of the Association assigns certain duties to its appointed officers who carry on the business and publishing responsibilities for the members. These duties are indicated in broad terms in Sections 3 and 4 of the By-Laws (J. Dairy Sci., 45:138. 1962.). This discussion deals with the organization of these duties.

Business functions are centered in the office of Secretary-Treasurer. In addition to the parliamentary duties of Secretary, he is required to perform or supervise these activities: send notices and collect dues, sell advertising space, sell back numbers, reprints, manuals, etc., collect monies due, pay out monies for expenses approved by the Executive Board, manage investments of reserve funds, account for all funds received and disbursed, and submit a budget to the Board to anticipate future income and expenditures.

Editorial functions are centered in the office of the Editor, who performs or supervises these activities: receiving and accepting manuscripts, editing manuscripts, soliciting special articles and papers, approving manuscripts and proofs, planning and supervising JOURNAL form, composition and content, writing and editing of popular sections, indexing, and communications.

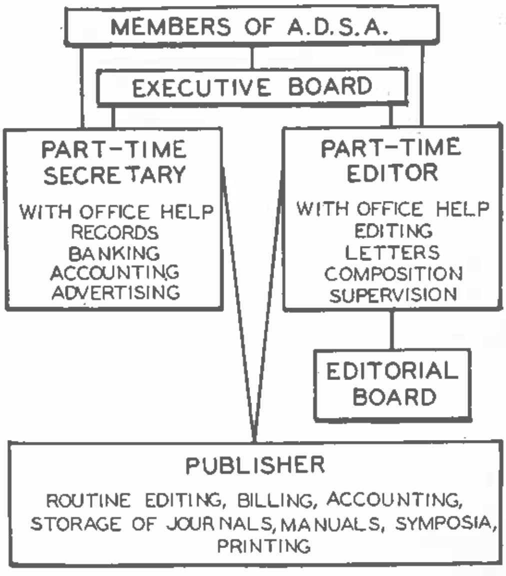
The Executive Board is responsible for maintaining continuous, efficient business and editorial functions within the limit of the budget it can approve. Over the years it has considered many methods to accomplish this objective, such as to contract with professional publishers, as the Association did in its early history, or to support a business office jointly with one or more professional societies. The American Society of Agronomy and the American Association of Cereal Chemists have been most cordial and helpful in exploring this last arrangement. The American Institute of Biological Sciences offers redactory and management services which resemble those provided by contracts with publishing concerns.

Future changes in the Association's membership and activities will affect management policy. These changes may be in numbers of members, diversification of interests, publication of more than one journal, permanent addition of new sections for economists and industrial managers, or other comparable additions of sections or divisions. Such changes may require some adjustments in methods of management, sources and amounts of revenue, and secretarial and editorial services.

With these facts in mind, the advisory Committee developed several plans of management with special emphasis on flexibility to meet different needs. Diagrams of lines of responsibility and communication within principal plans were prepared and submitted to the Executive Board in June, 1961, with a list of advantages and disadvantages of each.

Plans were numbered in the report so the same numbers will be used here. Only the more important objectives and contributions of each plan will be mentioned.

PLAN 1. This plan, used by the Association in 1960-61, gives the Executive Board direct supervision and control of Association affairs. Members with experience and prestige are elected by the Executive Board and serve as part-time officers and receive an honorarium



PLAN 1. Used until 1960-61 by A.D.S.A.

for their efforts. Such officers contribute professional attitude, knowledge of the Association, its members, and objectives. The term routine, used in the diagram, describes operations requiring technical skill only.

PLAN 2. In this plan a full-time Secretary-Treasurer sells advertising and supervises office help doing routine accounting and billing. He devotes more time than in Plan 1 to membership problems, contacts, and records.

The Executive Board voted in 1961 to consider this plan when the Association outgrows the services provided by Plan 6.

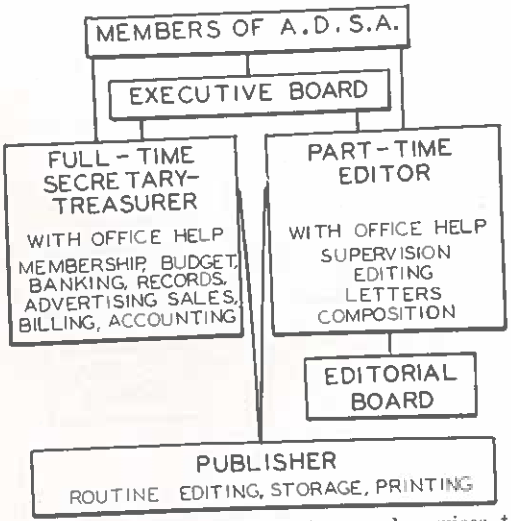
PLAN 3. This plan can be visualized by referring to the diagram of Plan 2. It merely expands the Secretary's responsibilities of Plan 2, to make him an executive Secretary-Treasurer or Manager. He would employ professional editors for the *routine* editing, composition, and production done by the publisher and Editor in Plans 1 and 2. In Plan 3 the part-time Editor, with the help of his Editorial Board, would be responsible only for passing on the scientific merit and content of papers; he might serve with or without honorarium.

This plan would meet future increases in publication and business responsibilities, give more flexibility in contracting for printing, concentrate office help in one business office, and let the scientific Editor function without production duties.

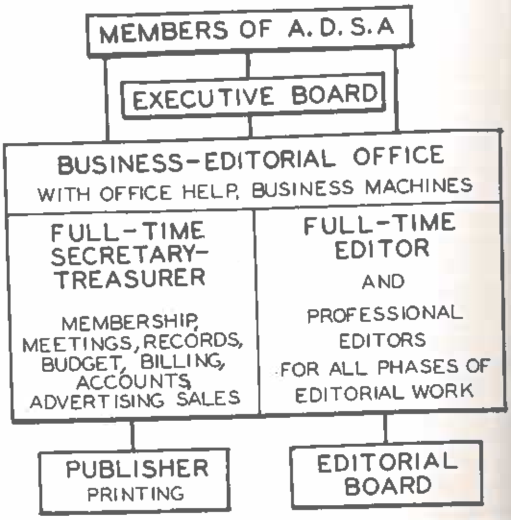
Another logical variation of Plan 3, which was only mentioned in Committee discussions, would place a full-time or executive Editor in charge of business operations and publishing instead of an executive Secretary. Then a recording Secretary would be needed only to record official actions of Board and Association, and to handle certain official communications.

PLAN 4. This plan is intended to meet the needs of a larger membership requiring more financial and publication services. It centralizes all business and editorial duties in one office, with both a full-time Secretary-Treasurer and Editor sharing the same business office, staff, and facilities.

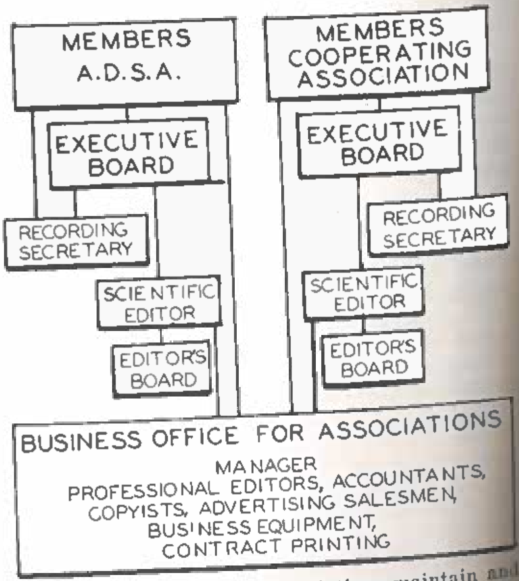
PLAN 5. This plan is diagrammed to show



PLAN 2. Used to handle increased services to membership by increased responsibility, activity, and supervisory work for a full-time Secretary-Treasurer.


MEMBERS OF A.D.S.A
EXECUTIVE BOARD
BUSINESS-EDITORIAL OFFICE
WITH OFFICE HELP, BUSINESS MACHINES
FULL-TIME SECRETARY-TREASURER
MEMBERSHIP, MEETINGS, RECORDS, BUDGET, BILLING, ACCOUNTS, ADVERTISING SALES
FULL-TIME EDITOR
AND
PROFESSIONAL EDITORS
FOR ALL PHASES OF EDITORIAL WORK
PUBLISHER
PRINTING
EDITORIAL BOARD

PLAN 4. For larger membership, greater responsibilities and services in management and publication.


MEMBERS A.D.S.A.
MEMBERS COOPERATING ASSOCIATION
EXECUTIVE BOARD
EXECUTIVE BOARD
RECORDING SECRETARY
RECORDING SECRETARY
SCIENTIFIC EDITOR
SCIENTIFIC EDITOR
EDITOR'S BOARD
EDITOR'S BOARD
BUSINESS OFFICE FOR ASSOCIATIONS
MANAGER
PROFESSIONAL EDITORS, ACCOUNTANTS, COPYISTS, ADVERTISING SALESMEN, BUSINESS EQUIPMENT, CONTRACT PRINTING

PLAN 5. Cooperating associations maintain and share common business and editorial services provided by a business manager and professional help.

how A.D.S.A. might share business, editorial facilities, and personnel with another association. Economies would be effected in office work, contracts for printing, and large-scale buying of supplies, such as paper in carload lots for printing. Such cooperative management would permit the Board to share responsibilities of choosing and supporting professional managers and editors, but it would also require the sharing of the constructive thinking and energies of such key persons in management. Some of the autonomy of A.D.S.A. management would be lost in choices of personnel and location of offices.

Plan 6. The Executive Board adopted this plan in June, 1961. It is not diagrammed because it is merely an extension of Plan 1. It assigns certain routine operations of the Secretary-Treasurer's and Editor's offices to the publisher, Garrard Press. These assignments, with the exception of printing, can be increased, decreased, or discontinued as desired, according to needs and relative costs. This plan enables each appointed officer to devote more attention to nonroutine, decision-making duties and activities for the Association, and still to retain supervision of his respective duties. Costs of operation vary with the amount of work delegated, but increases are offset in whole or in part by reduction in office expenses and the more productive efforts of the Secretary and Editor.

The writer is grateful for the assistance of the other members of the Business Management Committee of 1960-61 in presenting this brief description of their decisions to the members of the Association. A few copies of the complete report are in the chairman's hands and will be mailed as long as they last to those who can use them.

PRICE SCHEDULE FOR REPRINTS OF PAPERS THAT APPEAR IN THE JOURNAL OF DAIRY SCIENCE

H. F. Judkins, Secretary-Treasurer
32 Ridgeway Circle, White Plains, New York

The Executive Board, at the time of the Annual Meeting of the American Dairy Science Association at the University of Wisconsin, increased the price of reprints 25%, effective July 1, 1961. The new reprint schedule follows:

No. of reprints	Number of pages								
	2	4	8	12	16	20	24	28	32
	(Cost in dollars)								
50	17.50	20.00	36.25	51.25	67.50	78.75	97.50	115.00	125.00
100	20.00	22.50	41.25	61.25	77.50	92.50	112.50	132.50	145.00
200	22.50	28.75	51.25	76.25	97.50	117.50	143.75	162.75	185.00
300	28.00	33.75	62.50	91.25	117.50	143.75	173.75	205.00	226.25
400	30.00	40.00	72.50	107.50	137.50	170.00	205.00	241.25	266.25
500	33.75	45.00	83.75	122.50	157.50	195.00	236.25	277.25	306.25
600	37.50	51.25	93.75	137.50	177.50	221.25	266.25	313.75	346.25
700	41.25	56.25	105.00	153.75	197.50	246.25	297.50	350.00	387.50
800	45.00	62.50	115.00	168.75	218.75	272.50	328.75	386.25	427.50
900	48.75	67.50	126.25	185.00	238.75	298.75	358.75	422.50	467.50
1,000	57.25	73.75	136.25	200.00	258.75	323.75	390.00	458.75	507.50

If covers for reprints are desired, the cost of 50 covers will be $12.18, and for each additional 100 covers, the cost will be $8.75. Back copies of the Journal will cost $2 each.

The reprints are made from standing type within 30 days after the papers appear in the Journal. Requests for a few reprints of a paper should be sent to the authors, whose names and addresses appear with the title. The Secretary and the Editor's office do not keep supplies of the various reprints. Orders for large numbers of reprints should be sent to The Garrard Press, 510 North Hickory Street, Champaign, Illinois. These orders must be received within 30 days after the papers are published in the Journal; otherwise, the type will have been destroyed.

In case the original type has been destroyed, it is possible to supply reprints by a special photographic process, and their cost will be 50% more than the regular ones. For example, 100 reprints of 32 pages will cost $217.50.

It is hoped that the publication of this reprint schedule will make it easier for interested people to obtain reprints in any number desired and, at the same time, aid in disseminating useful information to the dairy and related industries.

The Journal of Dairy Science is copyrighted. Reproduction of complete papers from this Journal by any organization is not permitted. The reproduction of graphs, tables, and illustrations for books and other periodicals may be authorized by the Editor-in-Chief.